EFFECT OF GENTCHROMAN ON BIOCHEMICAL PROFILE, OVULATION AND OVARIAN SIZE

THESIS

FOR

MASTER OF SURGERY

(OBSTETRICS & GYNAECOLOGY)

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1992 DEEPA TANDON

C E R T I F I C A T E

This is to certify that the work entitled "EFFECT OF CENTCHROMAN ON BIOCHEMICAL PROFILE, OVULATION AND OVARIAN SIZE" which is being submitted as a thesis for M.S.(Obstetrics and Gynaecology) by Dr. Deepa Tandon has been carried out under direct supervision and guidance. The observations recorded have been periodically checked and verified by me .

She has put in the necessary stay in the department as per university regulations.

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INTRODUCTION

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Finally in this column of acknowledgement I wish to mention the persons who are an essential part of my life - my family and my husband. Their patience, affection and sacrifices have been a constant source of strength. No success of mine is apart from them and it is to them that I dedicate this work.

Dated: 31.7.91

Deepa Tandon.

(Deepa Tandon)

INTRODUCTION

Contraception is not only the prevention of conception but it is the control over conception. The world has been looking for a perfectly safe and effective contraceptive for thousands of years. During the centuries man has continuously evaluated various herbs and mechanical devices as means of controlling fertility with the result that many indigenous recipes or prescriptions were developed. However it is only in the last several decades that fertility control has become publically acceptable. Three factors have revolutionized the society's attitudes towards contraception, they are, the threat or a rapidly growing population, a desire for increased personal freedom and the development of oral contraceptives.

Attention was focussed on the development of a simple and effective oral chemical contraceptive and this search cluminated in the development of the oral contraceptive pill. It has been known for a considerable time that ovulation can be prevented by the administration of androgens, estrogens and progesterones.

As early as 1897, Beard and 1898 Zschokke observed that, the corpus luteum of the ovary that secretes progesterone is responsible for the inhibition of ovulation during pregnancy. Following the isolation

of progesterone in 1934, studies on several species of animals observed a similar effect of progesterone in ihhibiting ovulation. Evaluation of other steroids for their ability to inhibit ovulation had also begun. Parker and Bellerby(1926) later Burdik and Pincus observed that pregnancy could be prevented in rats and mice by injection of estrogens within one or two days after mating. Similar effects were observed with oral estrogens.

The first successful clinical trials of a combined oral contraceptive pill (estrogen and progesterone) were launched in Puerto Rico (1956) using a pill called Enovid. During the 1970's following their wide spread use, their side effects and difficulties in mass administeration became apparent. The search for an alternative oral contraceptive thus began.

The designing of molecules possessing estrogenic, antiestrogenic and antiprogestational properties which would possibily interfere primarily with intraovarian events appeared as a possible approach to the problem.

Diphenyl-ethylenes, triphenyl-ethylenes, methoxybenzefuran and naphtho-furan were shown to possess marked antifertility activity but due to undesirable side effects they were dropped from further studies. Later 3, 4 diphenyl chromenes and 3,4, diphenyl chromans were synthesized and their properties studied. Significant

antifertility activity was observed in the trans isomer of 3,4 diphenyl chroman, this led to the synthesis of centchroman (a trans chroman).

Centchroman is a non steroidal antifertility agent synthesized by the central Drug Research Institute Lucknow. Chemically it is 3,4 trans-2, 2-dimethyl-3 phenyl-4-(p-Beta-pyrrolidinoethoxy)-7 methoxy chroman with the chemical formula $C_{30}H_{35}O_3NHCl$. It is a white crystalline compound almost insoluble in water, its melting point is 165-166^{o}C and its $t\frac{1}{2}$ is 169 hours. The pure compound as well as its dosage form (Tablets) is stable even after storage upto 3 years.

Centchroman possesses estrogenic, antiesterogenic and antiprogesterogenic properties (Kamboj et al, 1971). It has been shown to possess properties as a post coital pill as well as an oral contraceptive.

Post coital administeration as a single oral dose within 24 hours of coitus prevented pregnancy in rats, mice (1.25 mg/kg) dogs and rhesus monkey (2.5 mg/kg). The antifertility effect was prompt and reversible (Kamboj, Shetty, Chandra Raj, Kar, 1977). The antifertility action in rats and monkeys appear to be at the level of the fallopian tubes and the uterus, the transport, fertilization and viability of ova is not disturbed, it is likely it prevents implantation by its multiple attributes such as estrogenic, antiestrogenic and antiprogestational activities.

Its pharmacological effects includes anti-inflammatory activity, incomplete adrenergic blockade, non specific spasmolytic activity and mild anorexogenic activity in dosages higher than the contraceptive dose. It is devoid of any pharmacological effects which may be reflected as side effects during its clinical use. It is found to be safe in animals and humans with an excellent therapeutic index.

Studies on healthy human females show that centchroman activates the hypothalmic pitutary axis to increase the serum levels of gonadotropins, there is an increase in the plasma total estrogens (120 mg/weekly dose). In spite of this an antiestrogenic effect is exerted by it, differentially on the vagina, cervix and endometrium. The drug does not seem to inhibit ovulation. It may have its contraceptive effect mainly due to its action on cervical mucous and endometrium, affecting sperm transport and implantation (Vaidya et al, 1977). It disturbs the delicate balance between endogenous estrogen and progesterone thereby interfering with uterine preparation for nidation and causing embryo endometrial asynchrony.

Contraceptive efficacy trials of centchroman at 60 mg post coital and 120 mg, 60 mg, 45 mg and 30 mg once a week schedules have revealed that it provides an acceptable pregnancy protection rate at the indicated dose in both the schedules.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Contraception is as ancient as history and the Ebers Papyrus, dating back to about 1550 BC was perhaps the first written reference to a medicated tampon designed to prevent conception.

As early as 1897 it was postulated by Beard that the corpus luteum of the ovary which secretes progesterone is responsible for inhibition of ovulation during pregnancy, this concept was supported by various studies performed on the corpus luteum during the ensuing twenty years (Pearl and Surface, 1914, Haberlandt, 1921, Smith, 1929, and Parkes, 1929). Followed by the isolation of progesterone from the corpus luteum in 1934 (Butenandt et al, 1934, Slotta et al, 1934, Allen and Winteresteiner, 1934, Hartmann and Wellstein, 1934) its physiological action were studied intensely. It became apparent that the hormone was capable of inhibiting ovulation in a variety of animal species.

Evaluation of othersteroids for their ability to inhibit ovulation had also begun. Parkes and Bellerby (1926) and later Burdick and Pincus (1935) observed that pregnancy could be prevented in rats and mice by injection of estrogens within one or two days after mating. Similar effects were observed with oral estrogens.

The first successful clinical trials of combined oral contraceptive pill(estrogen and progesterone)

were launched in Puerto Rico (1956), using a pill called Enovid). During the 1970's following wide spread use of combined oral pills, logistic difficulties in mass administration and the side effects associated with their use became apparent. This called for development of alternative contraceptive measures.

The designing of molecules possessing estrogenic and antiestrogenic (atypical estrogenic) and anti progestational properties which would possibly interfere with primarily infraovarian events appeared a possible approach to the problem. Since the demonstration of estrogenic activity of diphenylethylenes by Dodds et al (1938) and the estrogenic/antiestrogenic activity of triphenylethylenes (Segal and Nelson, 1958) it became clear that estrogenic activity is not highly structive specific.

Significant antifertility activity was found in 2-phenyl- (Beta-pyrolidiroethoxy) - phenyl-6-methoxy benzofuran and its related compounds when given within 24 hours of coitus in rhesus monkeys. These compounds showed a varying degree of estrogenic and antiestrogenic activity. They were however, dropped from further study due to undesirable side effects in toxicity studies.

Activity seen in 2,3, diphenyl benzofurans led to the synthesis and screening of a variety of related structural types and significant antifertility activity was observed in compounds like 3,4-diphenyl chromenes and

3,4, diphenyl chromans. The transforms of 3,4-diphenyl chromans were in general more active than the corresponding cis compounds. It is suggested that the differential effect of 2, alkyl substituent on biological activity is due to the change in configuration of the molecules, which has important implications in receptor binding. This led to the synthesis of centchroman(a trans chroman).

Centchroman is trans-2, 2-dimethyl-3-phenyl-4-p-(beta-pyrolidinoethoxy) phenyl-7-methoxy chroman, it is a non steroidal compound which was shown in experiments to prevent pregnancy in rats, mice, dogs and rhesus monkeys. Other effects of centchroman have also been explored and evaluated in variety of studies.

I. BIOLOGICAL EVALUATION

1. Antifertility efficacy

Centchroman at doses 0.25 mg/kg given orally to rats on days 1-5 post coitum caused 100% prevention of pregnancy. At a lower doses litter size was reduced although a few rats became pregnant. Accordingly 0.25 mg/kg is the minimum effective dose (ED 100) in the days 1-5 regime in rats. Likewise, in rats, a single feeding of centchroman at doses 1.25 and 2.5 mg/kg or any one of the days 1-4 post coitum was 100% effective in pregnancy prevention. The ED 100 in this regimen was 1.25 mg/kg. With lower doses, effectiveness decreased as the time lapsed post coitum increased.

Centchroman (1.25 mg/kg) as a single oral dose given 1, 2 or 3 days post coitum was 100% effective in preventing pregnancy in mice.

In dogs, centchroman administered once orally (2.5 to 5 mg/kg) or intramuscularly (1.5 and 2.5 mg/kg) 24 hours after mating caused 100% pregnancy prevention with no evidence of implantation.

During an 8 month clinical trial which rhesus monkey, 100% pregnancy prevention was observed with 2.5 mg/kg of centchroman given orally on the day following coitus.

The antifertility effect was promptly reversible on discontinuation of the treatment(Kamboj et al, 1977).

Contraceptive efficacy trials in women of reproductive age group as 60 mg post coital and 120 mg, 60,mg, 45 mg and 30 mg (oral) once a week schedules have revealed that centchroman provides an acceptable pregnancy protection rate. This being 4-5 pregnancies in 100 women years of use in the weekly dose schedule.

2. Endocrinological profile

a. Estrogenic activity :

In immature rats centchroman given orally(0.1, 0.25, 0.5, 1.25 and 2 mg/kg) or subcutaneously (1, 5, 10, 50 and 100 u gm/animal caused a significant increase in uterine weight. There was no indication of a dose response relationship. The uterotrophic potency was

40-50% that of esterone by oral route and 23-44% parenterally. The onset and duration of the uterotrophic activity was similar after centchroman and estrone treatments.

Doses of 1.5 mg and 2.0 mg/kg orally and 50-100 u gm by subcutaneously route induced vaginal opening and the smears presented pro estrus, and or estrus condition. Centchroman had a latent period in causing vaginal cornification compared with estrone.

Likewise in immature rhesus monkeys centchroman caused an increase in uterine weight and induced vaginal cornification. The uterotrophic potency being 64% that of esterone (Kamboj et al, 1977).

However in another study on immature mice centchroman given orally in a dose ranging from 5-200 u gm (total dose) produced a linear increase in uterine weight. Similar dose response relationship was observed with estradiol and mestranol. Vaginal cornification in ovarictomized mice after oral administration was 8-10 times less than that of mestranol and ethinyl estradiol (Munshi et al, 1977).

In all the above indices centchroman appear to be a weak estrogen. This is further supported by its failure to induce nidation in rats in a delayed implantation test. It also does not stimulate all the classic biochemical responses in the uterine fluid of rat like estrogen. Thereby revealing its weak or atypical estrogenicity (Kamboj et al, 1973).

b. Antiestrogenic activity :

Estrone (1 ugm/animal) given orally or parenterally to immature female rats, stimulated uterine weight and caused vaginal cornification. Simultaneous administration of centchroman diminished the uterotrophic response produced by esterone, the vaginal cornification was also suppressed. Centchroman is therefore antiestrogenic and is relatively more potent inhibiting uterotrophic activity of esterone than in preventing esterone induced vaginal cornification(Kamboj et al,1971).

In delayed implantation test however centchroman did not show any antiestrogenic activity. There was no effect on number of implantations after concurrent administration of progesterone (6 mg subcutaneously), estradiol dipropionate (1 ug subcutaneously) and centchroman(0.25 mg/kg oral) from days 8-12 of pregnancy. Centchroman per se did not induce implantation in this assay.

Joshi et al (1976) by in vitro studies indicated that centchroman completes with estrogen binding sites in the vagina, cervix and to a lesser extent the uterus of rabbits. This may explain the differential action of centchroman on the reproductive tract.

In human studies by Vaidya and co-workers(1977) distinct antiestrogenic effect of centchroman on vaginal cytohormonal pattern in the form of a depressed KPI has been demonstrated at 60 mg/week and 120 mg/week doses in spite of a markedly elevated circulating levels of

estrogen. The antiestrogenic effect was also reflected in the cervical mucous score at 120 mg/week dose but was not so noticable at 60 mg/week dose.

c. Progestational and antiprogestational activity :

Centchroman is devoid of progestational activity. It was not able to maintain pregnancy in ovariectomized rats at 25 mg/day (Nair et al, 1977).

Centchroman showed antiprogestational activity in Clauberg assay, delayed implantation and deciduoma induction assay (Kamboj et al, 1977). The antiprogestation activity however, is weak.

In Clauberg assay, oral administration of progesterone produced proliferative changes in the endometrium of immature rabbits. The administration of centchroman along with progesterone prevented the proliferative changes seen when the later alone was given.

In the delayed implantation test the administration of centchroman (0.25 mg/kg oral) in combination with progesterone (days 3-8 of pregnancy) completely prevented implantation.

Centchroman (Single oral dose of 1.25 mg/kg on day1 of pregnancy) prevented dediduoma formation in tubectomized, ovariectomized and traumatized rats treated with progesterone (2 mg/day) for 3 days (Kamboj et al, 1977).

In contrast it has been reported (Kumari et al, 1976) that centchroman does not interfere with the uptake of labelled progesterone by the target tissue. It also did not accelerate the metabolism or elimination of progesterone in the target tissue of ovariectomized rats.

Roy and Dutta (1977) supported the views that centchroman is devoid of anti progestational property. Adult female rats were ovariectomized on the third day of pregnancy and were treated with progesterone for 15 days. During the last 5 days of progesterone therapy 1 mg/kg of centchroman was given daily, centchroman failed to counteract the increase in weight and the total content of biochemical constituents of uterus caused by progesterone therapy.

d. Androgenic or antiandrogenic activity :

Centchroman is devoid of any androgenic or antiandrogenic properties (Kamboj et al, 1977).

3. Endocrine pharmacology

a. Effect on pitutary

Centchroman showed no effect on weight and gonadotrophin content of young male rat pitutary (Kamboj et al, 1977).

Studies on unilateral ovariectomized rats by Dutta and Roy (1977) indicated that centchroman in low doses has a gonadotrophin inhibitory effect but at a

higher dose level this is counteracted by some property which facilitates the release of gonadotrophins. This may probably be the antiestrogenic action of the compound and partly a direct positive feedback action on LH secretion.

Centchroman also has gonadotrophin modulating properties in women (Roy et al, 1976). It induces ovulation in anovulatory women and stimulates LH secretion in gonadal dysgenesis, patients with or without estrogen therapy. It has also been reported to stimulate the FSH and LH secretion by activation of the hypothalmic pitutary axis in normal male volunteers (Vaidya et al, 1976).

b. Effect on the thyroid :

Centchroman (1.25 mg/kg for 5 days, oral) had no significant effect on the thyroid weight, ^{131}I uptake and 'conversion rates' in immature female rhesus monkey. (Kamboj et al, 1977).

c. Effect on adrenal :

Centchroman caused an increase in the relative weight of the adrenal gland of male rats, which may be due to true adrenal hypertrophy (Das et al, 1977).

Centchroman did not influence the excretion rate of 24 hour urinary 17-OH-KGS in immature female rhesus monkey (at doses of 1.25 mg/kg oral x 5 days). Estrone however, caused a slight increase.

d. Effect on the foetus and fertility of the offspring :

Centchroman (1.25 mg and 2 mg/kg, oral) on days 5-7 of pregnancy in rats had no effect on the blastocyst on the newly implanted foetus. However, 17-32% less blastocysts implanted as compared to controls. A single feeding of centchroman on day 8th of pregnancy caused 30% foetal loss as against 14% in controls. Thus, centchroman administration, peri or post implantation, caused some foetal reabsorption.

Centchroman caused no abnormal genital development, masculinization or teratogenicity in the foetus and no detrimental effect on postnatal sexual development or fertility performance of the off spring (Kamboj et al, 1977).

II. MECHANISM OF ACTION

In experiments done by Kamboj et al (1977) the following observations were made :

a. Effect on ova

Centchroman (1.25 mg/kg, oral) on day 1 or pregnancy to rats did not impede transport and fertilization of ova. There was however, arrest of development in about 30% of the fertilized ova. The number of implantation were also reduced compared to controls.

b. Effect on deciduoma formation

Centchroman (1.25 mg/kg) single oral dose,

prevented deciduoma formation in tubectomized, ovariectomized and traumatized rats treated with progesterone (2 mg/day) for 3 days.

c. Effect of estrogen and progesterone on antifertility action

Centchroman interfered with the action of both estrogen and progesterone since neither of these hormones per se could induce implantation in compound treated rats.

d. Changes in the uterine mileu

Centchroman caused a lowering of the lactic acid and glycogen concentration in the uterine fluid of treated animals. This may be an important factors in its contraceptive action because blastocyst survival is unlikely in a mileu depleated of these things. Centchroman administration also reduced the protein content, alkaline phosphatase and acid phosphate activity in the uterine fluid.

Anand et al (1980) showed that centchroman administration decreased significantly the potassium concentration of uterine fluid on day 5 post coitus. Antiimplantation action of centchroman may be due to this decrease which there after may increase the negative membrane potential on endometrium causing the negatively charged endometrium to repel the blastocyst bearing a similar charge.

Studies on human female volunteers (Vaidya, Joshi, Mehra, Razi, Batrabet, Joshi, Sheth and Devi) showed that centchroman does not inhibit ovulation although it may delay it but it exerts its contraceptive effect mainly due to its action on the cervical mucous and endometrium affecting sperm transport and implantation.

III. GENERAL PHARMACOLOGY AND TOXICITY

The effect of centchroman (doses ranging from contraceptive dose to 1/5 LD 50) on nictating membrane, CNS, CVS, respiration, isolated tissue, diuretic and anti-inflammatory activities were studied in different animals and compared with standard drugs. Centchroman was found to have an anti-inflammatory activity, incomplete alpha adrenergic blockade, non specific spasmolytic activity and mild anorexigenic effect at high doses(Mukerjee, Sethi, Shrivastava, Roy, Nityanand and Mukerjee, 1977). Centchroman possessed excellent therapeutic index and is devoid of any pharmacological effect which may be reflected as a side effect during clinical use as a contraceptive.

a. Chronic Toxicity

Chronic toxicity studies were carried out with centchroman on young adult male and female albino rats (doses of 6, 25, 12.5 and 25 mg/kg, orally, once daily for 7 months). Similar studies were also carried out in rhesus monkey. No adverse effects were revealed on

haematology, biochemistry and histopathology of different organs. Thus centchroman was shown to be devoid of any toxicity upto twenty times the contraceptive dose (Mukerjee et al, 1977).

A study on the effect of long term centchroman treatment on reproductive organs of female rats (Mehrotra, 1979) showed it to produce no untoward effect on the genital tract.

Non steroidal estrogens have been reported (Mukerjee et al, 1977) to produce an increase in liver weight, particularly in high doses which is considered to be due to non specific stress reaction.

b. Acute Toxicity

Acute toxicity was determined by administering graded dose of the compound to groups of mice and rats. LD50 determined by intraperitoneal route in mice was 400 mg/kg. Oral LD 50 was determined in both mice and rats and was more than 1500 mg/kg. Since the oral contraceptive dose in mice and rats is 1.25 mg/kg. The compound has a very high margin of safety.

Centchroman was devoid of any significant gross effects or CNS effects.

IV. TERATOGENICITY

In a study to evaluate the influence on prenatal development of foetus in mice and rabbits, centchroman at

doses 20, 40 and 80 times of 100% antifertility dose (ED 100) was given orally to pregnant mice and rabbits during period of organogenesis. It did not cause abortion or congenital anomalies. There were no defects in skeleton or other organs of foetus. The reabsorption rate in mice was within normal limits, only 50 mg/kg, the higher dose showed a higher rate of 26%. In rabbit it was 40% as against 12-14% in controls of both the species. The litter size and weight in both species was comparable to controls. In both the animals no abnormality developed in the offspring postnatally upto 6 weeks.

V. CLINICAL PHARMACOLOGY STUDIES

Centchroman was subjected to clinical pharmacological studies in healthy male and female volunteers as a double blind non cross over trial(Chandra et al, 1977).

In single dose tolerance study with 40 volunteers, centchroman in doses increasing from 5 mg to 320 mg was well tolerated with no side effects, abnormal physical or laboratory findings.

In multiple dose study with 28 volunteers, centchroman was given as 60 or 120 mg once daily for 30 days. There was no change in vital signs even at the highest dose used. Laboratory tests were normal in all the groups. In none of the volunteers hypotension was observed as might be expected from its non specific anti-spasmolytic activity observed in animals(Chak et al)

In two subjects (one of each group) a delay in menstruation of 35 to 60 days was observed. Two cases had scanty periods. Symptoms recorded were headache, letharginess, nausea, bodyache, giddiness, fever and burning mictutition.

In another study on the effect of centchroman in 10 healthy women of child bearing age with history of normal menstrual cycle conducted by Vaidya et al (1977), Centchroman was given at two dose levels of 60 mg and 120 mg/week. Increased length of cycle was noticed in all cases, probably due to lengthening of the follicular phase. Centchroman at the mentioned doses did not seem to inhibit ovulation but may delay it. The contraceptive effects were mainly exerted on cervical mucous and endometrium.

In a study of the effect of centchroman in normospermic and oligospermic individuals by Roy et al (1977). There was no alteration in liver and kidney function tests in both individuals during therapy.

In contraceptive efficacy trials with women volunteers, centchroman has been found to provide good pregnancy protection in 60 mg post coital and 120, 60, 45 and 30 mg once a week schedules. The only side effect observed was a variable delay in the onset of mensus in some cycles. The prolonged cycles were random and not restricted to any individual or any particular cycle

of treatment, most cases resumed normal cycles while still on treatment. No other drug related side effects were observed. Physical and vaginal examination revealed no abnormality during the trial. Haematological examination, lipid profile, platelet aggregation and organ function tests remained within normal limits. At 30 mg/ week dose no ovarian abnormalities were found during laproscopy or ultrasonography.

Babies born to user failures developed normally. The effect of centchroman was readily reversible and subsequent pregnancies were normal.

MATERIAL AND METHODS

MATERIAL AND METHODS

The study was carried out in the department of Obstetrics and Gynaecology and Department of Pathology, Maharani Laxmi Bai Medical College, Hospital, Jhansi over a period of one year.

1. VOLUNTEER SELECTION

Cases attending the out patients department of Obstetrics and Gynaecology formed the material of the study. Normal healthy women of reproductive age group (20-35 years) with normal clinical and gynaecological history were registered after obtaining their informed consent.

Criteria for inclusion and exclusion

At the time of registration :

a. Volunteers having a normal menstrual cycle pattern.

b. Post abortal cases presenting after at least one normal cycle.

c. No woman with a history of jaundice, severe anaemia, diabetes mellitus, hypertension or any other major illness was included.

d. No pregnant or lactating women were selected.

e. Volunteers who had not used steroidal contraceptives for at least 3 months prior to enrollment.

f. Women agreeing to use centchroman as the only method of contraception for the study period.

HISTORY

All patients were in the reproductive age group (20-35 years) with normal menstrual history. Complete obstetrical and menstrual history was taken.

GENERAL EXAMINATION

Thorough general examination of all patients was done and the pulse, blood pressure, pallor, oedema and weight of the patient were noted.

SYSTEMIC EXAMINATION

Examination of the cardiovascular system, respiratory system and abdomen was done.

LOCAL EXAMINATION

1. Per speculum examination (P/S) was done to inspect the cervix and the vaginal walls.
2. Per vaginum examination (P/V) - was done to note position and size of the uterus and its appendages. The ovaries were examined to note any enlargement and whether palpable or not.

2. SCHEDULE OF USE OF CENTCHROMAN

Centchroman 30 mg tablets, oral, twice a week for the first three months and then once a week schedule was followed. First tablet was given on the first day of the ensuing mensus after registration and there after every Sunday and Wednesday irrespective of mensus day.

The tablets were continued even if subsequent menses were delayed. From the fourth month onwards the patients were given one tablet (30 mg) every Sunday irrespective of the menses day.

3. CRITERIA FOR CONTRACEPTIVE EVALUATION :

The volunteers were asked to follow the schedule of drug use strictly. Any lapse on the part of volunteer was recorded.

Any pregnancy occurring due to tablet ommission or non schedule use was classified as patient failure(PF). The contraceptive efficacy was determined from the number of method failure(MF) pregnancies during the study period.

4. FOLLOW UP

Clinical observations and findings of laboratory investigations were recorded before drug administration and subsequently at periodic intervals (Post drug).

LABORATORY TESTS

To assess the effect of centchroman on biochemical profile assessment of the following laboratory parameters (from sample of urine, blood and serum) was done before administration of the drug and subsequently at +3, +6 and +12 months of drug intake.

1. Urine analysis - Sugar and albumin.
2. Haemoglobin - mg/dl
3. Serum bilirubin- mg/dl

4. Serum G.P.T. (I.U.).

5. Blood urea (mg/dl).

6. Serum creatinine (mg/dl).

7. Serum lipids : a. Cholesterol (mg/dl).

   b. Triglycerides (mg/dl).

   c. High density lipoprotein cholesterol (mg/dl).

Urine Albumin

Detection of proteins was done using the heat coagulation test.

Urine Sugar

Qualitative determination of sugar in urine was done by Benedict's reagent.

Haemoglobin

It was estimated by the Sahli's haemoglobinometer. A definite quantity of blood is converted to acid haematin by the addition of N/10 HCl. The colour is then matched against the standard provided in the haemoglobinometer.

Serum Bilirubin

Estimation of serum bilirubin was done by the Van Den Berg method (1913) whose basis is the reaction of bilirubin in its soluble form with the diazo reagent to form a purple colour which is measured colorimetrically.

S.G.P.T.

Estimation is based on the principle that the S.G.P.T. in the serum by enzymatic action on ketoglutaric acid and aspartic acid converts them to glutamic acid and pyruvic acid which when treated with 2-4-dinitrophenyl hydrazine in alkaline medium give a brown colourd hydrazone which is measured colorimetrically.

Serum Creatinine

Estimation is by modified version of Brod et al (1948) method. Creatinine reacts with picric acid in the alkaline medium and a red colour develops (over 85% colour is due to creatinine) it is measured calorimetrically.

Serum Lipids

Serum total cholesterol, serum triglycerides and high density lipoproteins were estimated with standard diagnostic kits supplied by Ethnor India Ltd..

a. Serum Total Cholesterol (STC) :

Principle of estimation is that cholesterol reacts with a hot solution of ferric perchlorate, ethyl acetate and sulphuric acid and gives a lavender coloured complex which is measured calorimetrically (Wyanbenga and Pillegi method, 1970).

b. Serum Triglycerides (STG) :

Principle of estimation by the acetyle acetone

method is that, the glycerol is liberated from the fatty acids in the triglycerides after saponification, it is then oxidized by sodium metaperiodate to formaldehyde which is directly proportional to the amount of triglyceride.

c. High Density Lipoproteins (HDL) :

Principle of estimation is that the HDL cholesterol fraction is separated by using a precipitating reagent. The precipitants contain chylomicrons, VLDL, LDL which are removed by centrifugation. The supernatant contains HDL-c which is estimated by HDL-c colour reagent which gives purple coloured complex which is measured colorimetrically.

Special tests

1. Vaginal smear examination.
2. Cervical mucous study.
3. Endometrial aspiration cytology.
4. Ultrasonography.

1. Vaginal Smear Examination :

The vaginal smear was prepared in mid cycle (14/15th day) and one day premenstrually (27th day of normal 28 day cycle) in the pretreatment cycle and subsequently on the same day of each treatment cycle.

Smear fixation :

The vaginal smear was taken from the lateral

vaginal wall before any gynaecological examination and the secretions were spread on clean labelled glass slides (two in number). The slides were immediately transfered to a mixture of equal parts of 95% alcohol and ether for fixation.

Staining :

The papanicoloau's method of staining was used. The slides were passed through the following solutions in a sequential manner :

a. 80% alcohol (1/2 min) b. 70% alcohol (1/2 min.)

c. 50% alcohol (1/2 min.) d. Distilled water (1/2 min.)

e. Harris haematoxylin (3 min.) nuclear staining. f. Distilled water (1/2 min.)

g. 0.25% aquous HCl (6 dips) h. Running water (6 min.)

i. Distilled water (1/2 min.) j. 50% alcohol (1/2 min.)

k. 70% alcohol (1/2 min.) l. 80% alcohol (1/2 min.)

m. 95% alcohol (1/2 min.) n. Orange G-6 (1 1/2 min.)

o. 95% alcohol (1/2 min.)
p. 95% alcohol (1/2 min.)
} Separate containers.

q. EA-15 (1 1/2 min.) (Eosin Azure)

r. 95% alcohol (1/2 min.)
s. 95% alcohol (1/2 min.)
t. 95% alcohol (1/2 min.)
} separate containers

u. Absolute alcohol (1/2 min.) v. Xylol alcohol (1/2 min.)

w. Xylol (1/2 min.)

The nuclei are stained with Harris haemotoxylin and the cytoplasm with orange G and alcoholic light green-Bismark Brown Eosin (Protoplasmic stain) solution.

Cytology :

The superficial cells, intermediate cells and the parabasal cells were studied and the maturation index and the Karyopyknotic index(KPI) were determined.

The smear was studied for evidence of trichomonas and monilial infection.

Trichomonas are frequently present in vaginal smears, their presence is not associated with hormonal factors. They appears as small, shapeless and usually pale grey spots of variable size scattered between the cells.

Monilia albicans : The fungi can be identified in vaginal smears by their oral or rounded spores and mycelia which appears as irregular pale granular threads of variable length and showing constrictions - Candida attacks the glycogen rick epithelium.

Superficial cells : They are large delicate polyhedral cells with sharply defined cell borders which may be irregular or indented. The nucleus is pyknotic. The cytoplasm may be eosinophilic or cynophilic.

Intermediate cells : They are medium sized cells with extreme variability in size. The cytoplasm is mostly cynophilic and the nuclei are large and vesicular.

Parabasal cells : They are rounded cells with cynophilic cytoplasm and a relatively large rounded central nucleus of delicate but distinct structure .

Maturation Index

It expresses the level of cellular maturation attained at the time of exfoliation. Two hundred cells are counted in different fields and the number of superficial, intermediate and parabasal cells is determined. It is expressed in terms of hundred cells.

Maturation Index (MI) = Parabasal/intermediate/ superficial cells.

Karyopyknotic Index

It denotes the percentage of cells in the smear with nuclear pyknosis. Two hundred cells are counted and those with pyknotic nuclei are expressed in percentage.

The karyopyknotic index under the influence of estrogen reaches a maximum about the 14th or 15th day of the normal cycle. In general there is a good co-relation between the estrogen and pregnediol excretion in the urine and the karyopyknotic index curves.

Cervical Mucous Study

Cervical mucous was studied for spinnbarkeit and fern test on 14/15th day and premenstrually (27th day of a normal 28 day cycle) in the pretreatment cycle and subsequently on similar days in each treatment cycle.

Spinnbarkeit :

A drop of cervical mucous was put on a glass slide and this was covered with another glass slide. Both the slides were slowly separated and the distance upto which the slides could be separated without breaking the mucous thread was noted in centimeters.

Fern Test

A drop of mucous was spread on a clean glass slide to make a thick smear and was air dried. It was then examined under low power for the ferning pattern of the mucous.

Endometrial Aspiration Cytology

Smear of endometrial aspirate was prepared one day premenstrually (27th day of a 28 day cycle) in the pretreatment cycle and subsequently on same days of each treatment cycle.

Collection of material :

A uterine cannula made of steel and a 20 cc glass syringe was used for aspiration from the endometrial cavity.

Technique of Endometrial aspiration :

With the patient in lithotomy position, (after evacuating the bladder) a pervaginal examination was done, posterior vaginal wall was then retracted by Sim's speculum and anterior vaginal wall by the anterior vaginal

wall retractor. Anterior cervical lip was held by volselleum. Endometrial aspiration cannula was passed into uterine cavity. Negative pressure was created by a 20 ml glass syringe and the negative pressure maintained till the tip of the aspiration cannula was withdrawn to the level of internal os (in cases where no aspirate was obtained 0.5 ml of normal saline solution was injected into uterine cavity through the cannula and the procedure repeated).

A drop of aspirated material was placed on a glass slide and the smear immediately fixed in equal parts of 95% alcohol and ether.

Staining was done by the papanicoloau's method, similar to that used for vaginal cytology.

Cytology :

Two main types of cells are recognizable.

Endometrial Epithelial Cells

They appear in aggregates or clumps and are low columner with sparse cynophilic cytoplasm which readily disintegrates therefore distinct cell borders are often absent. Nuclei are coarsely granular appearing dark and structurally irregular.

Endometrial Stromal Cells

They include a wide variety of morphologically diverse cells shed singly or in groups. They include

syncytial reticular cells or histocytes, macrophages, monocytes and lymphocytes. They lack the regular structure or epithelial cells.

Cyclical endometrial cellular changes

Involve the shape, size and structure of the nuclei (Krauss, 1951), mitosis is regarded as a sign of proliferative phase. In the secretory phase cytoplasmic vacuolation and cellular degeneration appear(Boschann, 1957). These observations are however, not uniform.

Ultrasonography

Ovarian size was measured by ultrasonography employing a real time sector scanner using a frequency of 3.5 MHz (Philips SDR 1550 x p).

A pulse of ultrasound is produced by the pizoelectric effect of the crystals in its transducer, the passage of this beam of ultrasound through the body generates echoes when it encounters interfaces. The intensity of the echo varying with the density of the tissue encountered. The returning echoes are converted back into electrical signals in the transducer and perceived on screen as images which are displayed in different shades of grey. Cystic areas appear dark and more solid areas appear whiter.

Scanning is done in the supine position with a full urinary bladder in order to provide an acoustic

window into the pelvis. Serial scans in the transducer being angled towards the side walls of the pelvis.

The ovaries are almond shaped mobile organs due to the variable laxity of their attachments by the mesovarium and the ovarian ligaments. The ovarian size varies according to the phase of the menstrual cycle and the patients endocrinological status. Maximum length is usually less than 4 cm though thin ovaries may be longer.

The stimation of ovarian valume was done using the ellipsoid formula :

$$\text{Volume}(cm^3) = \text{Long axis} \times \text{Short axis} \times \text{AP axis} \times 0.5$$

OBSERVATIONS

OBSERVATIONS

In the present work, the effect of centchroman a new non steroidal oral contraceptive on biochemical profile, ovulation and ovarian size in 17 females of the reproductive age group was studied.

During the first visit of the subject to the department, thorough general examination was done. Basal sample of blood was withdrawn for various biochemical tests as mentioned earlier. The basal ovarian size was noted by ultrasonography, vaginal cytology and endometrial aspiration cytology was done in the desired phase (mid cycle and premenstrually). The women were given the drug according to schedule and blood and urine tests and ovarian measurements were done at +3, +6 and +12 months, vaginal cytology and cervical mucous study was done in the appropriate days in each cycle.

The observations made are mentioned in the form of various tables.

A. GENERAL CHARACTERISTICS

TABLE I : Distribution of cases according to age (maximum age recorded was 34 years).

Sl. No.	Age groups (years)	No.of cases	Percentage
1.	20 - 22	5	29.41
2.	23 - 25	4	23.53
3.	26 - 28	5	29.41
4.	29 - 31	2	11.77
5.	32 - 34	1	5.88
	TOTAL	17	

Five (29.41%) cases each, were in age group of 20-22 and 26-28 years. Four (23.53%) cases were in the age group of 23-25 years. Two (11.77%) were in the age group of 29-31 years and only 1(5.88%) case was in age group of 32-34 years (Table I).

TABLE II : Distribution of cases according to parity.

Sl. No.	Parity	No.of cases	Percentage
1.	1	2	11.77
2.	2	9	52.94
3.	3	5	29.41
4.	4	1	5.88
	TOTAL	17	

Table II shows that 9 cases (52.94%) were second para, 5 cases (29.41%) were third para, 2 cases (11.77%) were of parity one, and 1 case (5.88%) was fourth para.

TABLE III : Distribution of cases according to initial cycle length.

Sl. No.	Cycle length (days)	No.of cases	Percentage
1.	Upto 24	-	-
2.	25 - 35	17	100.00
3.	36 - 45	-	-
4.	45 & above	-	-
	TOTAL	17	

Table III shows that the length of cycle in all (100%) cases was between 25-35 days.

TABLE IV : Distribution of cycles during treatment according to cycle length.

Sl. No.	Cycle length (days)	No.of cases	Percentage
1.	Upto 24	-	-
2.	25 - 35	90	89.11
3.	36 - 45	8	7.92
4.	45 and +	3	2.97
	TOTAL	101	

Table IV shows that in 89.11% cycles, the cycle duration was 25-35 days. In 7.92% cycles the duration was 36-45 days. Cycle length was greater than 45 days in 2.97% of the cycles during treatment.

TABLE V : Distribution of cases according to their duration of flow in pre treatment cycle.

Sl. No.	Cycle duration (days)	No.of cases	Percentage
1.	1 - 2	1	5.88
2.	3 - 4	8	47.06
3.	5 - 6	5	29.41
4.	7 6	3	17.65
	TOTAL	17	

Prior to treatment 8(47.06%) cases had duration of flow of 3-4 days, 5 cases (29.41%) had a duration of flow 5-6 days. Three cases (17.65%) had a duration of flow of more than six days. Only 1 case (5.88%) had a duration of flow of 1-2 days (Table V).

TABLE VI : Distribution of cycles during treatment according to their duration of flow.

Sl. No.	Duration of flow (days)	No.of cycles	Percentage
1.	1 - 2	37	36.63
2.	3 - 4	38	37.62
3.	5 - 6	21	20.79
4.	7 6	5	4.95
	TOTAL	101	

Table VI shows that 37.62% of treatment cycles had a duration of flow of 3-4 days, 36.63% were of 1-2 days flow, 20.79% had a duration of flow of 5-6 days and only 4.95% had a flow lasting greater than 6 days.

TABLE VII : Distribution of cycles during treatment according to amount of flow.

Sl. No.	Amount of flow	No.of cycles	Percentage
1.	No change	62	61.39
2.	Increased	1	0.99
3.	Decreased	38	37.62
	TOTAL	101	

The amount of menstrual flow during treatment cycles was noted as same, increased or decreased as compared with pretreatment cycles.

Table VII showed that the amount of flow remained unchanged in 61.39% of cycle. In 37.62% of cycles the quantity of flow was decreased. Only one cycle(0.99%) showed an increase in the amount of flow.

TABLE VIII-A : Effect of centchroman on weight at 0 Vs +3 months of use.

Sl. No.	Weight in kg		Difference in weight
	0 value	+3 value	
1.	47	47.5	0.5
2.	36	37	1
3.	58	58	-
4.	67	67	-
5.	48	48	-
6.	46	46	-
7.	45	45	-
8.	46	47	1
9.	42	42	-
10.	42	42	-
11.	40	39	- 1
12.	45	45	-
13.	50	50	-
14.	45	46	1
15.	40	40	-
16.	35	35	-

't' = 1.12, d.f. = 15, p $>$ 0.05

At +3 months, four patients showed an increase in weight and one patient showed a decrease in weight whereas in 11 patients no change in weight occurred. Thease changes were not statistically significant. (Table VIII-A).

At +6 months, six patients showed an increase in weight and two patients showed a decrease. There was no change in weight of one patients. These changes

were not statistically significant (Table VIII-B).

TABLE VIII-B : Effect of centchroman on weight at 0 Vs +6 months of use.

Sl. No.	Weight in kg.		
	0 value	+6 value	Difference
1.	47	47.5	0.5
2.	36	37	1
3.	58	58.5	0.5
4.	67	67.5	0.5
5.	48	50	2
6.	46	46	-
7.	45	44	- 1
8.	46	47	1
9.	46	45	- 1

't' = 1.22, d.f. = 8, p $>$ 0.05

TABLE VIII-C : Effect of centchroman on weight at 0 Vs +12 months of use.

Sl. No.	Weight in kg.		
	0 value	+12 value	Difference
1.	47	48	1
2.	36	38	2
3.	58	60	2
4.	46	45	- 1
5.	35	35	-

't' = 1.38, d.f. = 4, p $>$ 0.05

At 12 months of use of centchroman three patients showed an increase in weight, one showed a decrease and one patient showed no change in weight. These differences were not statistically significant. (Table VIII-C).

CLINICAL OBSERVATIONS

Physical and pervaginal examination of all volunteers during the trial could not diagnose any abnormality. All findings were within normal limits.

B. BIOCHEMICAL TESTS

Distribution of cases according to findings of urine analysis in pretreatment and treatment cycles is shown in table IX-A and IX-B.

TABLE IX-A :

Urine albumin	0		+3		+6		+12	
	No.	%	No.	%	No.	%	No.	%
Nil	13	76.47	15	93.75	7	77.78	5	100.00
Traces	4	23.53	1	6.25	2	22.22	-	-
Present	-	-	-	-	-	-	-	-

TABLE IX-B

Urine sugar	0		+3		+6		+12	
	No.	%	No.	%	No.	%	No.	%
Nil	16	94.12	15	93.75	9	100.00	4	80.00
Traces	-	-	-	-	-	-	1	20.00
Present	1	5.88	1	6.25	-	-	-	-

At +3 months 16 patients turned up whereas at ±6 months only 9 cases were available for follow up and at +12 months only 5 cases remained in follow up. Therefore the percentage of cases showing a particular finding of urine examination has been calculated taking the respective number of patients that were followed up.

No case showed the presence of urine albumin at 0 months, 13 cases (76.47%) and 4 cases (23.53%) showed absence and traces of urine albumin respectively.

At +3 months, 93.75% (15 cases) showed urine albumin nil and 6.25% (1 case) showed traces of urine albumin. No case showed the presence of albumin in urine.

At +6 months, 77.78%(7 cases) showed urine albumin nil and 22.22(2 cases) showed traced of urine albumin. In no case was urine albumin present.

At +12 months 100% (5 cases) showed urine albumin as nil (Table IX-A).

At 0 month 94.12% (16 cases) showed no urine sugar, 5.88% (1 case) who showed the presence of sugar in urine and no case showed traces of urine sugar (Table IX-B).

At +3 months 93.75% (15 cases) showed urine sugar nil. 6.25%(1 case) showed presence of sugar in urine and no case showed traces.

At +6 months all(9 caaes) 100% showed sugar to be nil.

At +12 months 80%(4 cases) showed no urine sugar and 20%(1 case) showed traces of urine sugar.

Table X-A showed that there was an increase in haemoglobin of 8 cases at +3 months as compared with values of 0 months and two patients showed a fall in haemoglobin. There was no change in haemoglobin in remaining 6 cases. The changes were statistically insignificant.

TABLE X-A : Effect of centchroman on Haemoglobin gm% at 0 Vs +3 months.

Sl. No.	Haemoglobin gm%		
	0 value	+3 value	Difference
1.	10.0	10.0	-
2.	12.5	12.5	-
3.	13.0	13.0	-
4.	12.0	12.2	0.2
5.	09.0	9.8	0.8
6.	11.0	11.4	0.4
7.	9.0	10.0	1.0
8.	11.2	11.8	0.6
9.	11.0	10.6	- 0.4
10.	12.0	12.0	-
11.	11.0	11.0	-
12.	11.0	12.0	1.0
13.	9.0	9.4	0.4
14.	12.0	12.0	-
15.	10.0	11.0	1.0
16.	10.0	8.0	- 2.0

't' = 0.999, d.f. = 15, p 70.05

TABLE X-B : Effect of centchroman on haemoglobin gm% 0 Vs + 6 months.

Sl. No.	Haemoglobin gm%		
	0 value	+6 value	Difference
1.	10.0	10.0	-
2.	12.5	12.8	0.3
3.	13.0	13.6	0.6
4.	12.0	13.0	1.0
5.	9.0	12.0	3.0
6.	11.0	12.0	1.0
7.	9.0	10.0	1.0
8.0	11.2	12.4	1.2
9.	11.0	10.0	- 1.0

't' = 2.197, d.f. = 8, p 70.05

At +6 months, 7 patients showed an increase in haemoglobin, one patient showed a decrease and only one patient showed no change in haemoglobin. These changes were not statistically significant.

TABLE X-C : Effect of centchroman on haemoglobin gm%
0 Vs +12 months.

Sl. No.	Haemoglobin gm%		
	0 value	+12 value	Difference
1.	10.0	11.0	1.0
2.	12.5	13.0	0.5
3.	13.6	13.6	-
4.	10.0	9.0	- 1.0
5.	11.0	11.6	0.6

't' = 0.64, d.f. = 4, p $>$ 0.05

At +12 months, 3 patients showed an increase and one patients showed a decrease in haemoglobin values as compared with initial values. In one patient value remained unchanged. These changes were not statistically significant.

TABLE XI-A : Effect of centchroman on serum bilirubin levels at 0 Vs +3 months.

Sl. No.	Serum bilirubin		
	0 value	+3 value	Difference
1.	0.2	0.2	-
2.	0.1	0.1	-
3.	0.3	0.3	-
4.	0.2	0.2	-
5.	0.2	0.2	-
6.	0.1	0.1	-
7.	1.0	1.0	-
8.	0.2	0.2	-
9.	0.3	0.3	-
10.	1.2	1.4	0.2
11.	1.0	1.2	0.2
12.	0.8	0.7	- 0.1
13.	0.9	0.9	-
14.	0.6	0.5	- 0.1
15.	0.5	0.3	- 0.2
16.	1.0	0.8	- 0.2

't' = 0.46, d.f. = 15, p $>$ 0.05

The difference in serum bilirubin was not statistically significant.

TABLE XI-B : Effect of centchroman on serum bilirubin levels 0 Vs +6 months.

Sl. No.	Serum bilirubin (mg%).		
	0 value	+6 value	Difference
1.	0.2	0.2	-
2.	0.1	0.1	-
3.	0.3	0.8	0.5
4.	0.2	0.2	-
5.	0.2	0.1	- 0.1
6.	0.1	0.1	-
7.	1.0	1.0	-
8.	0.2	0.2	-
9.	1.0	1.2	0.2

't' = 0.81, d.f. = 8, p $\gtrless$ 0.05

Changes in serum bilirubin at 6 months were statistically insignificant.

TABLE XI-C : Effect of centchroman on serum biliruin levels 0 Vs +12 months.

Sl. No.	Serum bilirubin (mg%)		
	0 value	+12 value	Difference
1.	0.2	0.4	0.2
2.	0.1	0.4	0.3
3.	0.3	0.6	0.3
4.	1.0	0.9	- 0.1
5.	1.0	0.7	- 0.3

't' = 0.67, d.f. = 4, p $\gtrless$ 0.05

On applying the test of significance the changes observed were not significant.

TABLE XII-A : Effect of centchroman on SGPT levels 0 Vs +3 months.

Sl. No.	S.G.P.T. 0 value	S.G.P.T. +3 value	Difference
1.	10	10	-
2.	7	5	-2
3.	8	8	-
4.	5	5	-
5.	12	12	-
6.	6	5	-1
7.	14	14	-
8.	6	6	-
9.	8	8	-
10.	12	12	-
11.	11	11	-
12.	14	12	-2
13.	13	14	+1
14.	12	11	-1
15.	10	12	2
16.	11	10	-1

't' = 1.00, d.f. = 15, p $>$ 0.05

Changes observed were found to be statistically insignificant.

TABLE XII-B : Effect of centchroman on SGPT levels at 0 Vs +6 months.

Sl. No.	S.G.P.T. 0 value	S.G.P.T. +6 value	Difference
1.	10	11	11
2.	7	7	-
3.	8	8	-
4.	5	5	-
5.	12	11	-1
6.	6	5	-1
7.	14	14	-
8.	6	6	-
9.	11	13	2

't' = 0.359, d.f. = 8, p $>$ 0.05

On applying the test of significance the changes observed were not significant.

TABLE XII-C : Effect of centchroman on SGPT levels 0 Vs +12 months.

Sl. No.	S.G.P.T.		
	0 value	+12 value	Difference
1.	10	10	-
2.	7	8	1
3.	8	6	-2
4.	11	10	-1
5.	11	13	2

't' = 0, d.f. = 4, p γ0.05

Differences between 0 value and +12 values were found to be statistically insignificant.

TABLE XIII-A : Effect of centchroman on blood urea 0 months Vs +3 months.

Sl. No.	Blood urea		
	0 value	+3 value	Difference
1.	21	22	1
2.	22	20	-2
3.	28	25	-3
4.	31	30.6	-0.4
5.	29	27	-2
6.	21	21	-
7.	30	31	1
8.	15	15	-
9.	32	32	-
10.	36	38	2
11.	20	28	8
12.	40	36	-4
13.	23	23	-
14.	33	30	-3
15.	20	20	-
16.	20	24	4

't' = 0.14, d.f. = 15, p γ0.05

Differences observed in blood urea were not significant statistically.

TABLE XIII-B : Effect of centchroman on blood urea 0 Vs +6 months.

Sl. No.	Blood urea		
	0 value	+6 value	Difference
1.	21	22	1
2.	22	20	-2
3.	28	25	-3
4.	31	31	-
5.	29	28	-1
6.	21	22	1
7.	30	30	-
8.	15	15	-
9.	20	24	4

't' = 0, d.f. = 8, p $\nearrow$ 0.05

On applying the significance test, the changes were found to be not significant statistically.

TABLE XIII-C : Effect of centchroman on blood urea 0 Vs +12 months.

Sl. No.	Blood urea		
	0 value	+12 value	Difference
1.	21	20	-1
2.	22	22	-
3.	28	26	-2
4.	20	24	4
5.	20	22	2

't' = 0.56, d.f. = 4, p $\nearrow$ 0.05

Changes in blood urea at +12 months were not statistically significant.

TABLE XIV-A : Effect of centchroman on serum creatinine levels 0 Vs +3 months.

Sl. No.	Serum Creatinine		
	0 value	+3 value	Difference
1.	1.2	1.4	0.2
2.	0.4	0.3	-0.1
3.	0.6	0.5	-0.1
4.	0.1	0.1	-
5.	0.3	0.3	-
6.	0.1	0.1	-
7.	0.3	0.3	-
8.	0.2	0.4	0.2
9.	0.2	0.2	-
10.	1.0	1.1	0.1
11.	1.5	1.5	-
12.	1.5	1.5	-
13.	1.0	1.0	-
14.	1.5	1.3	-0.2
15.	1.0	0.9	-0.1
16.	1.0	1.0	-

't' = 0, d.f. = 15, p $>$ 0.05

Difference shown in this table XIV-A were not statistically significant.

TABLE XIV-B : Effect of centchroman on serum creatinine levels 0 Vs +6 months.

Sl. No.	Serum Creatinine		
	0 value	+6 value	Difference
1.	1.2	1.2	-
2.	0.4	0.3	-0.1
3.	0.6	0.5	-0.1
4.	0.1	0.1	-
5.	0.3	0.4	0.1
6.	0.1	0.1	-
7.	0.3	0.3	-
8.	0.2	0.4	0.2
9.	1.5	1.4	-0.1

't' = 0, d.f. = 8, p $>$ 0.05

On applying the test of significance the changes observed were found to be insignificant.

TABLE XIV-C : Effect of centchroman on serum creatinine levels 0 Vs +12 months.

Sl. No.	Serum Creatinine		
	0 value	+12 value	Difference
1.	1.2	1.0	-0.2
2.	0.4	0.4	-
3.	0.6	0.5	-0.1
4.	1.0	1.1	0.1
5.	1.5	1.3	-0.2

't' = 1.37, d.f. = 4, p $>$ 0.05

The changes were found to be insignificant statistically.

TABLE XV-A : Effect of centchroman on serum cholesterol 0 Vs +3 months.

Sl. No.	Serum cholesterol		
	0 value	+3 value	Difference
1.	200	190	-10
2.	180	186	6
3.	192	192	-
4.	210	210	-
5.	200	206	6
6.	206	206	-
7.	175	175	-
8.	120	120	-
9.	160	160	-
10.	170	170	-
11.	220	218	- 2
12.	130	135	5
13.	140	142	2
14.	135	132	- 3
15.	185	185	-
16.	150	158	8

't' = 0.71, d.f. = 15, p $>$ 0.05

On applying the test of significance the changes observed were found to be insignificant statistically.

TABLE XV-B : Effect of centchroman on serum cholesterol levels 0 Vs +6 months.

Sl. No.	Serum cholesterol		
	0 value	+6 value	Difference
1.	200	190	-10
2.	180	188	8
3.	192	196	4
4.	210	212	2
5.	200	200	-
6.	206	204	- 2
7.	175	175	-
8.	120	120	-
9.	200	190	-10

't' = 0.005, d.f. = 8, p $>$ 0.05

On applying the test of significance the changes were found to be insignificant.

TABLE XV-C : Effect of centchroman on serum cholesterol levels 0 Vs +12 months.

Sl. No.	Serum Cholesterol		
	0 value	+12 value	Difference
1.	200	200	-
2.	180	188	8
3.	192	190	- 2
4.	150	156	6
5.	200	190	-10

't' = 0.13, d.f. = 4, p $>$ 0.05

On applying the test of significance the changes were found statistically insignificant.

TABLE XVI-A : Effect of centchroman on serum triglycerides levels 0 Vs +3 months.

Sl. No.	Serum triglyceride		
	0.value	+3 value	Difference
1.	95	100	5
2.	110	112	2
3.	100	98	- 2
4.	120	120	-
5.	90	92	2
6.	86	88	2
7.	110	110	-
8.	92	92	-
9.	82	82	-
10.	92	92	-
11.	60	60	-
12.	68	70	2
13.	90	90	-
14.	96	86	-10
15.	71	72	1
16.	88	96	8

't' = 0.68, d.f. = 15, p $>$ 0.05

On applying the test of significance the changes were found to be insignificant.

TABLE XVI-B : Effect of centchroman on serum triglyceride levels 0 Vs +6 months.

Sl. No.	Serum triglycerides		
	0 value	+6 value	Difference
1.	95	102	7
2.	110	112	2
3.	100	100	-
4.	120	120	-
5.	90	92	2
6.	86	88	2
7.	110	110	-
8.	92	82	-10
9.	95	100	5

't' = 0.56, d.f. = 8, p $>$ 0.05

Changes observed in serum triglycerides were found to be statistically insignificant.

TABLE XVI-C : Effect of centchroman on serum triglycerides levels 0 Vs +12 months.

Sl. No.	Serum Triglycerides		
	0 value	+12 value	Difference
1.	95	100	5
2.	110	112	2
3.	100	100	-
4.	88	93	5
5.	95	102	7

't' = 2.74, d.f. = 4, p $>$ 0.05

On applying the test of significance, the changes were not significant statistically.

TABLE XVVI-A : Effect of centchroman in serum high density lipoprotein cholesterol 0 Vs +3 months.

Sl. No.	HDL Cholesterol		
	0 value	+3 value	Difference
1.	40	42	2
2.	51	55	4
3.	63	59	-4
4.	70	72	2
5.	50	55	5
6.	49	43	-6
7.	67	67	-
8.	42	42	-
9.	55	55	-
10.	64	60	-4
11.	70	65	-5
12.	48	50	2
13.	57	56	-1
14.	67	63	-4
15.	48	48	-

't' = 0.69, d.f. = 14, p $>$ 0.05

Differences in HDL cholesterol were not statistically significant.

TABLE XVII-B : Effect of centchroman on serum HDL cholesterol 0 Vs +6 months.

Sl. No.	HDL cholesterol		
	0 value	+6 value	Difference
1.	40	42	2
2.	51	56	5
3.	63	58	-5
4.	70	71	1
5.	50	55	5
6.	49	43	-6
7.	67	67	-
8.	42	39	-3

't' = 0.42, d.f. = 7, p $>$ 0.05

On applying the test of significance the changes were found to be statistically insignificant.

TABLE XVII-C : Effect of centchroman on serum HDL cholesterol 0 Vs +12 months.

Sl. No.	HDL cholesterol		
	0 value	+12 value	Difference
1.	40	41	1
2.	51	52	1
3.	63	60	-3

Sample size in this case was too small to apply the test of significance accurately.

C. SPECIAL TEST

I. Ovarian size :

Ultrasonographic measurement of ovarian size was done at 0, +3, +6 months of drug use. Ovarian size was expressed in terms of ovarian volume using the ellipsoid formula:

Ovarian volume = L x AP x T x 0.5 cc where

L = Longitudinal axis, T = Transverse axis,

AP= Anteroposterior axis.

TABLE XVIII-A : Effect of centchroman on ovarian size 0 Vs +3 months.

Sl. No.	Ovarian volume		
	0 value	+3 value	Difference
1.	7.74	6.31	-1.43
2.	4.16	7.70	3.54
3.	8.75	26.60	17.85
4.	13.76	14.36	0.60
5.	4.79	5.03	0.24
6.	2.96	2.90	-0.06
7.	6.60	9.25	2.65
8.	6.27	8.71	2.44
9.	4.58	2.99	-1.59
10.	3.53	3.63	0.10
11.	3.80	5.30	1.50
12.	4.26	5.69	1.43
13.	4.13	2.81	-1.32
14.	7.90	12.39	4.49
15.	7.51	7.76	0.25

't' = 1.677, d.f. = 14, p $>$ 0.05

On applying the test of significance the changes observed were not found to be statistically significant.

It was observed that maximum ovarian enlargement at +3 months (17.85) was present in the case whose cycle was prolonged by 168 days.

TABLE XVIII-B : Effect of centchroman in ovarian size 0 Vs +6 months.

Sl. No.	Ovarian size		
	0 value	+6 value	Difference
1.	7.74	6.24	-1.5
2.	4.16	26.99	22.83
3.	8.75	6.6	-2.15
4.	13.76	21.10	7.34
5.	4.79	7.00	2.21
6.	2.96	3.41	0.45
7.	7.26	7.49	0.23

't' = 1.265, d.f. = 6, p $\gtrsim$ 0.05

On applying the test of significance the changes in ovarian size observed at 6 months of use were not found to be significant statistically.

It was observed that the maximum ovarian enlargement (22.83cc) at 6 months was observed in the case having a cycle prolonged upto 171 days.

Ovarian volume had receded in the case showing maximum enlargement at +3 months.

D. VAGINAL CYTOLOGY

The Karyopyknotic index was determined in the vaginal smear prepared midcycle i.e. 14/15 day and premenstrually i.e. 27th day of cycle. The maturation index corresponded to changes in KPI by increase or decrease in superficial cells.

In the pretreatment cycle 13 cases (86.67%) had KPI more than 50% and 2 cases (23.08%) had KPI between 41-50%.

In the first treatment cycle 6 cases (46.15%) showed KPI between 21-30%, 3 cases (23.08%) had KPI greater than 50%, 2 cases (25.38%) had KPI between 31-40% and KPI was between 40-50% and 11-20% in one case (7.69%) each.

In the second treatment cycle 7 cases (58.33%) had KPI between 21-30%, 4 cases (33.33%) had KPI between 11-20% and 1 case (8.33%) had KPI between 31-40%.

In the third treatment cycle, 8 cases (66.67%) had KPI between 11-20%, 3 cases (25%) had KPI between 21-30% and remaining 1 case (8.33%) had KPI between 41-50%.

In the fourth treatment cycle, 7 cases (63.64%) had KPI ranging from 11-20%, 3 cases (27.27%) had KPI in between 21-30% and 1 case (9.09%) had KPI between 0-10%.

In the 5th treatment cycle, 5 cases (71.43%) had KPI in the range of 11-20% and 1 case each (14.29%) with KPI in range of 0-10% and 41-50%.

TABLE XIX-A : Distribution of cases according to Karyopyknotic index on 14/15th day in pretreatment and various treatment cycles.

Sl. No.	Cycle No.	No. of cases followed up	0-10%		11-20%		21-30%		31-40%		41-50%		750%	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1.	0	15	1	-	-	-	-	-	-	-	2	13.33	13	86.67
2.	1	13	-	-	1	7.69	6	46.15	2	15.38	1	7.69	3	23.08
3.	2	12	-	-	4	33.33	7	58.33	1	8.33	-	-	-	-
4.	3	12	-	-	8	66.67	3	25.00	-	-	1	8.33	-	-
5.	4	11	1	9.09	7	63.64	3	27.27	-	-	-	-	-	-
6.	5	7	1	14.29	5	71.43	-	-	-	-	1	14.29	-	-
7.	6	6	1	16.67	4	66.67	-	-	1	16.67	-	-	-	-
8.	7	2	-	-	1	50.00	-	-	-	-	1	50.00	-	-
9.	8	2	1	50.00	-	-	1	50.00	-	-	-	-	-	-
10.	9	2	-	-	1	50.00	1	50.00	-	-	-	-	-	-
11.	10	2	-	-	2	100.00	-	-	-	-	-	-	-	-
12.	11	2	-	-	2	100.00	-	-	-	-	-	-	-	-
13.	12	1	-	-	1	100.00	-	-	-	-	-	-	-	-

In the 6th treatment cycle, 4 cases(66.67%) had KPI in range of 11-20%, 1 case(16.67%) had KPI in the range of 0-10% and rest one case in the range of 31-40%.

In the 7th treatment cycle 1 case(50%) had KPI in the range of 11-20% and one case(50%) had KPI in the range of 41-50%.

In the 8th treatment cycle 1 case(50%) had KPI between 0-10% and 1 case(50%) had KPI between 21-30%.

In the 9th treatment cycle 1 case(50%) had KPI between 11-20% and 1 case(50%) had KPI between 21-30%.

In the 10th, 11th and 12th cycle, all (100%) cases had KPI between 11-20%.

Three patients in the pretreatment cycle showed presence of trichomonas in the vaginal smear and were treated for the same.

TABLE XIX-B : Distribution of cases according to KPI on 27th day of cycle in pretreatment and various treatment cycles.

Sl. No.	Cycle No.	No. of cases followed up	0-10%		11-20%		21-30%		31-40%	
			No.	%	No.	%	No.	%	No.	%
1.	0	15	3	20.00	8	53.33	4	26.67	-	-
2.	1	13	5	38.46	4	30.77	1	7.69	3	23.08
3.	2	11	6	54.55	4	36.36	-	-	1	9.09
4.	3	11	7	63.63	2	28.57	2	28.57	-	-
5.	4	10	5	50.00	2	20.00	3	30.00	-	-
6.	5	5	2	40.00	2	40.00	1	20.00	-	-
7.	6	5	4	80.00	1	20.00	-	-	-	-
8.	7	1	1	100.00	-	-	-	-	-	-
9.	8	2	1	50.00	1	50.00	-	-	-	-
10.	9	2	2	100.00	-	-	-	-	-	-
11.	10	2	2	100.00	-	-	-	-	-	-
12.	11	1	1	100.00	-	-	-	-	-	-
13.	12	1	1	100.00	-	-	-	-	-	-

In the pretreatment cycle, (53.33%) 8 cases had KPI in range of 11-20%, (26.67%) 4 cases had KPI between 21-30% and (20%) 3 cases had it between 0-10%.

In the first treatment cycle, (38.46%) 5 cases had KPI between 0-10%, (30.77%) 4 cases had KPI between 11-20%, (23.08%) 3 cases had KPI greater than 30% and (7.69%) 1 case had KPI ranging from 21-30%.

In the 2nd treatment cycle, (54.55%) 6 cases had KPI ranging from 0-10%, (36.36%) 4 cases had KPI between 11-20%, and (9.09%) 1 case had KPI greater than 30%.

In the 3rd treatment cycle, (63.63%) 7 cases had KPI between 0-10%, (28.57%) 2 cases each were in the range of KPI of 11-20% and 21-30%.

In the fourth treatment cycle (50%) 5 cases had KPI between 0-10% and (30%) 3 cases had KPI in range of 21-30% whereas (20%) 2 cases had it in range of 21-30%.

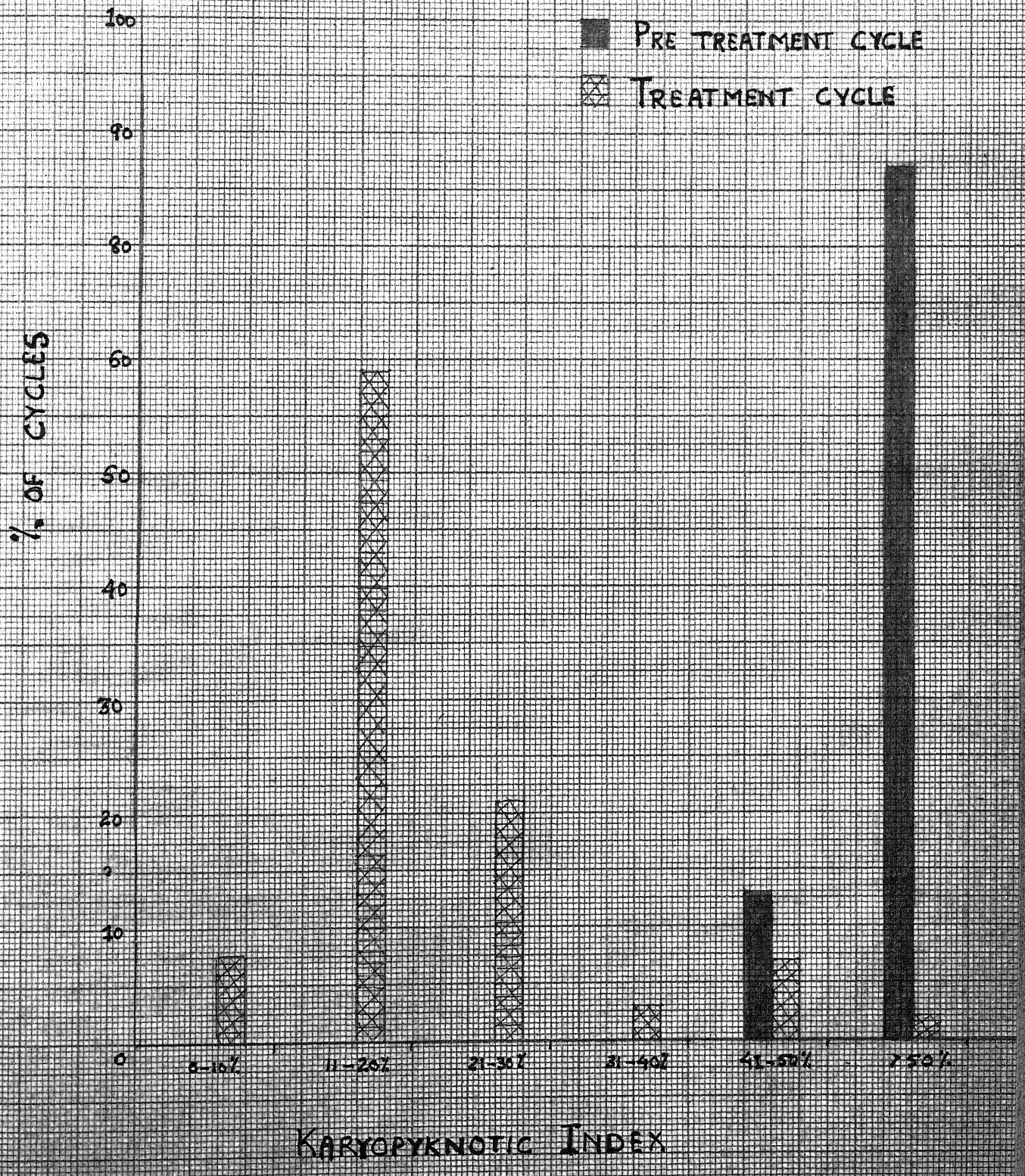
In the 5th treatment cycle (40%) 2 cases each were in the ranges of KPI of 0-10% and 11-20% and (20%) 1 case had KPI between 21-30%.

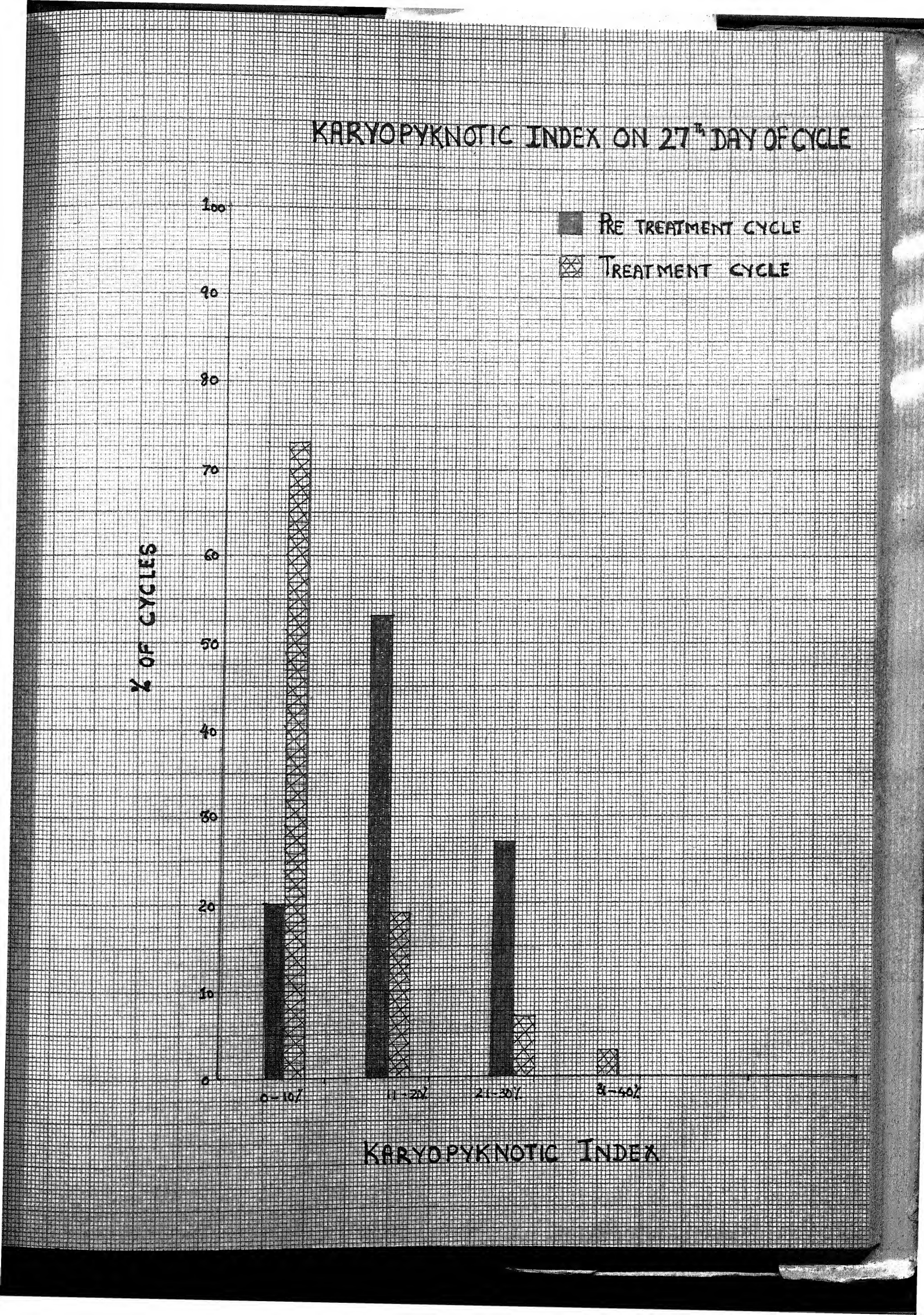
In the sixth treatment cycle (80%) 4 cases had KPI between 0-10% and (20%) 1 case had KPI between 11-20%.

In the 7th treatment cycle,100% cases had KPI in between 0-10%.

In the 8th treatment cycle (50%) 1 case had KPI in between 0-10%.and another (50%) 1 case had KPI between 11-20%.

KARYOPYKNOTIC INDEX ON 14/15th DAY OF CYCLE




KARYOPYKNOTIC INDEX ON 27TH DAY OF CYCLE
PRE TREATMENT CYCLE
TREATMENT CYCLE
% OF CYCLES
100
90
80
70
60
50
40
30
20
10
0
0-10%
11-20%
21-30%
31-40%
KARYOPYKNOTIC INDEX

In the 9th, 10th, 11th and 12th treatment cycles all (100%) cases had KPI in between 0-10%.

III. CERVICAL MUCOUS TEST

1. Spinnbarkeit test.

2. Fern test.

TABLE XX-A : Distribution of cases according to spinnbarkeit test at 14/15th day of pretreatment and treatment cycles.

Sl. No.	Cycle No.	No. of cases followed up	0-5 cm		6-10 cm		7 10 cm	
			No.	%	No.	%	No.	%
1.	0	15	-	-	2	13.33	13	86.67
2.	1	13	-	-	10	76.92	3	23.08
3.	2	12	1	8.33	10	83.33	1	8.33
4.	3	12	2	16.67	10	83.83	-	-
5.	4	11	2	18.18	9	81.82	-	-
6.	5	7	-	-	7	100.00	-	-
7.	6	6	1	16.67	4	66.67	1	16.67
8.	7	2	-	-	2	100.00	-	-
9.	8	2	1	50.00	1	50.00	-	-
10.	9	2	-	-	2	100.00	-	-
11.	10	2	-	-	2	100.00	-	-
12.	11	1	-	-	1	100.00	-	-
13.	12	1	-	-	1	100.00	-	-

In the pretreatment cycle (86.67%) 13 cases showed a spinnbarkeit of greater than 10 cm and (13.33%) 2 cases had spinnbarkeit in between 6-10 cm.

In the 1st treatment cycle, (76.92%) 10 cases showed thread formation in the range of 6-10 cm while only (23.08%) 3 cases showed spinnbarkeit of greater than 10 cm.

In the 2nd treatment cycle, (83.33%) 10 cases showed spinnbarkeit of 6-10 cm whereas (8.33%) 1 case had spinnbarkeit of 0-5 cm and another (8.33%) 1 case had spinnbarkeit greater than 10 cm.

In the 3rd treatment cycle (83.33%) 10 cases had thread formation of 6-10 cm and (16.67%) 2 cases had thread formation in range of 0-5 cm.

In the 4th treatment cycle (81.82%) 9 cases had a thread stretcheable to 6-10 cm and (18.18%) 2 cases had spinnbarkeit of 0-5 cm.

In the 5th treatment cycle all the 7 cases(100%) had spinnbarkeit of 6-10 cms.

In the 6th treatment cycle(66.67%) 4 cases had 6-10 cm thread formation whereas only (16.67%) 1 case had thread of 0-5 cm and another (16.67%) 1 case had thread more than 10 cm.

In the 7th treatment cycle total (100%) 2 cases showed spinnbarkeit of 6-10 cms.

In the 8th treatment cycle out of 2cases, (50%) 1 case had thread formation of 0-5 cm and another (50%) 1 case had thread of 6-10 cm.

In the 9th, 10th and 11th cycle (100%) 2 cases had thread stretcheable to 6-10 cm.

In the 12th treatment cycle only one cases was followed and it showed thread of 6-10 cm.

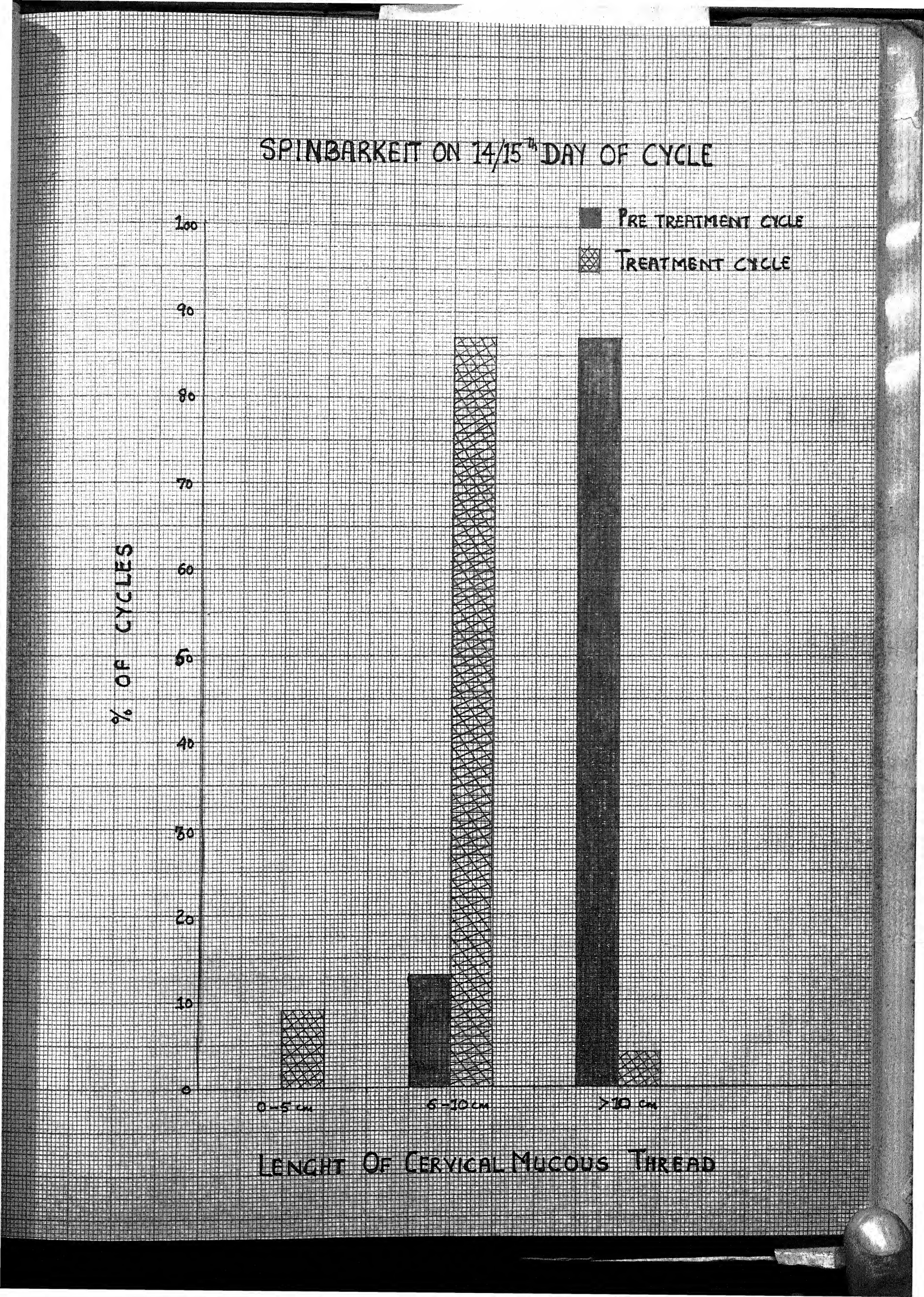

SPINBARKEIT ON 14/15TH DAY OF CYCLE
PRE TREATMENT CYCLE
TREATMENT CYCLE
% OF CYCLES
100
90
80
70
60
50
40
30
20
10
0
0-5 cm
6-10 cm
>10 cm
LENGHT OF CERVICAL MUCOUS THREAD

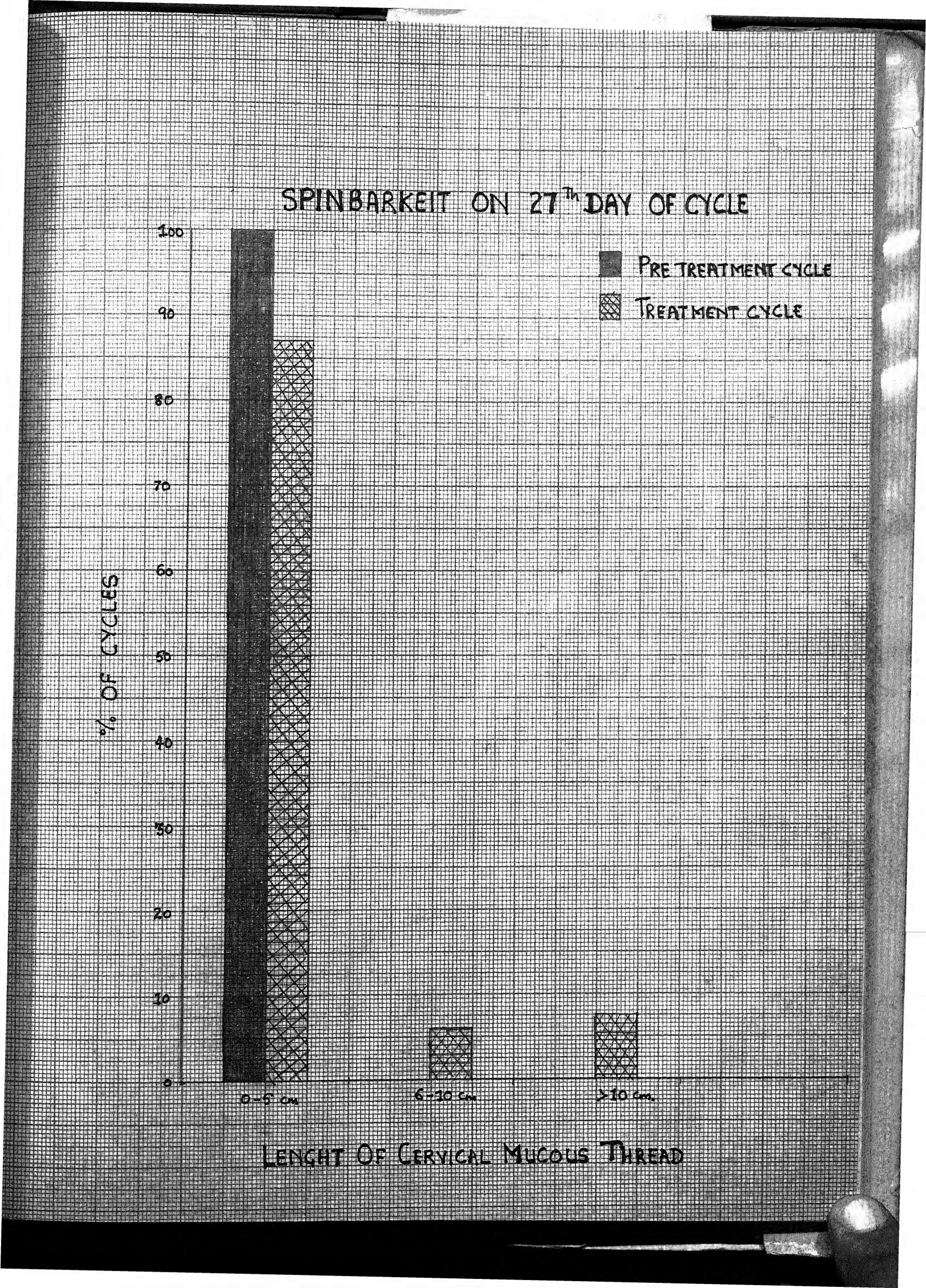
TABLE XX-B : Distribution of cases according to spinnbarkeit test on 27th day of pretreatment and treatment cycles.

Sl. No.	Cycle No.	No.of cases followed up	0-5 cm		6-10 cm		7 10 cm	
			No.	%	No.	%	No.	%
1.	0	15	15	100.00	-	-	-	-
2.	1	13	9	69.23	-	-	4	30.77
3.	2	11	9	81.82	1	9.09	1	9.09
4.	3	11	10	90.91	1	9.09	-	-
5.	4	10	6	60.00	3	30.00	1	10.00
6.	5	5	3	60.00	1	20.00	1	20.00
7.	6	5	4	80.00	-	-	1	20.00
8.	7	1	1	100.00	-	-	-	-
9.	8	2	2	100.00	-	-	-	-
10.	9	2	2	100.00	-	-	-	-
11.	10	2	2	100.00	-	-	-	-
12.	11	1	1	100.00	-	-	-	-
13.	12	1	1	100.00	-	-	-	-

In the pretreatment cycle all 15 cases (100%) showed a spinnbarkeit of 0-5 cm.

In the first treatment cycle, (69.23%) 9 cases showed a thread of 0-5 cm length and (30.77%) 4 cases showed a spinnbarkeit of greater than 10 cm.

In the 2nd treatment cycle, (81.82%) 9 cases showed a spinnbarkiet between 0-5 cm, (9.09%) 1 case had a thread of 6-10 cm and another (9.09%) 1 case had the thread formation greater than 10 cm.


SPINBARKEIT ON 27TH DAY OF CYCLE
PRE TREATMENT CYCLE
TREATMENT CYCLE
% OF CYCLES
100
90
80
70
60
50
40
30
20
10
0
0-5 cm
6-10 cm
>10 cm
LENGHT OF CERVICAL MUCOUS THREAD

In the 3rd treatment cycle, 9 (90.91%) cases showed spinnbarkeit of 0-5 cm and remaining (9.09%) 1 case had a thread of 6-10 cm of length.

In the 4th treatment cycle out of 10 cases, (60%) 6 cases had a thread of 0-5 cm, (30%) 3 cases had a thread of 6-10 cm and remaining (10%) 1 case had spinnbarkeit of greater than 10 cm.

In the 5th treatment cycle out of 5 cases, (60%) 3 cases had thread formation between 0-5 cm, (20%) 1 case had thread of 6-10 cm and another (20%) 1 case had greater than 10 cm.

In the 6th treatment cycle, (80%) 4 cases had a thread of 0-5 cm and (20%) 1 case had the thread of greater than 10 cm.

In the 7th treatment cycle, (100%) 1 case had spinnbarkeit of 0-5 cm.

In the 8th, 9th and 10th treatment cycle, 2 cases (100%) had spinnbarkeit of 0-5cm.

In the 11th and 12th treatment cycle only 1 case each was followed up and both (100%) showed a thread of 0-5 cm.

2. Fern Test

Ferning was graded according to Macdonald's recommendations i.e. 0 = no fern formation, + = slightly positve(weak), ++ = moderately positive and +++ = strongly positive.

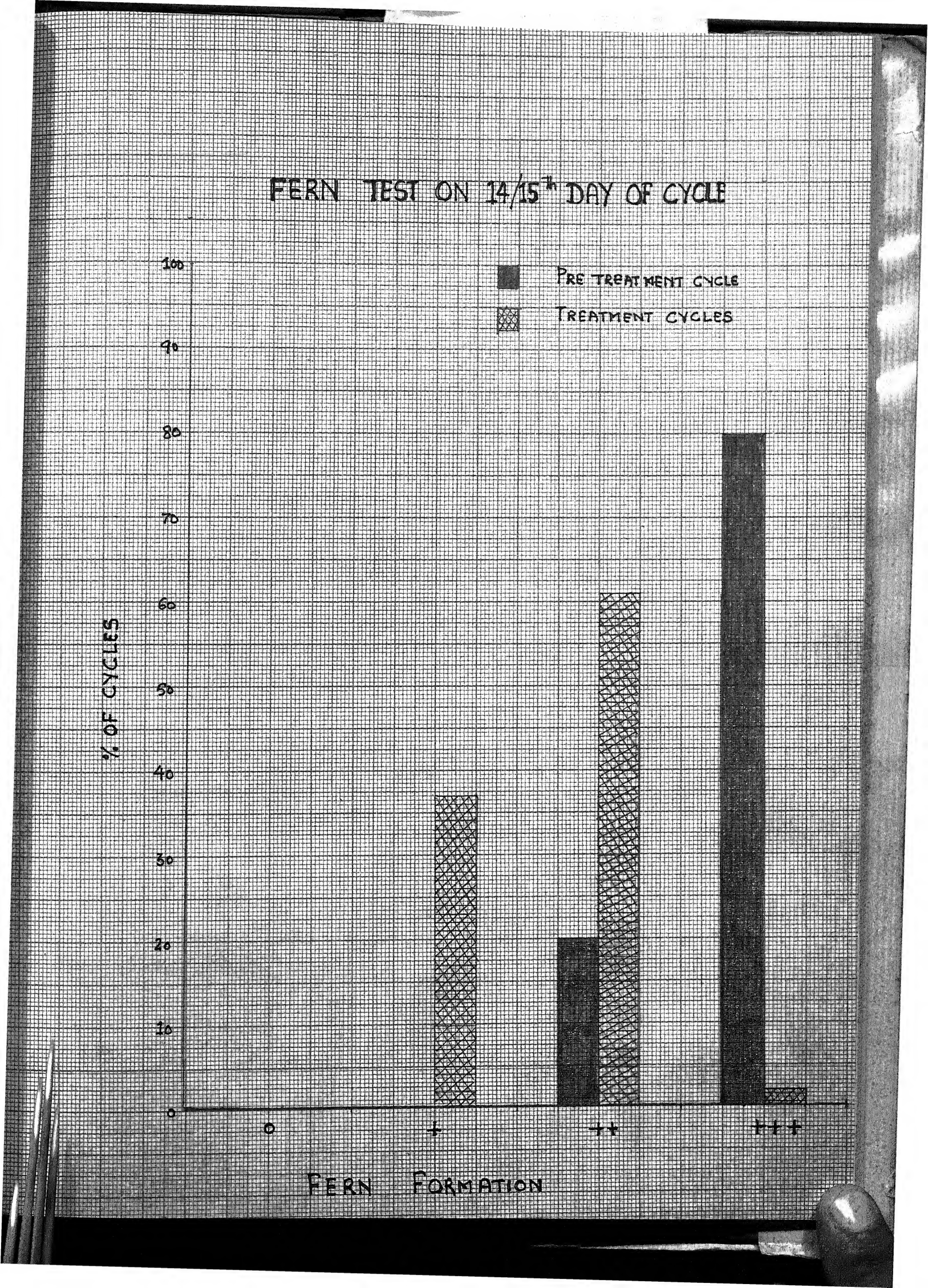
TABLE XXI-A : Distribution of cases according to fern test at 14/15th day of pretreatment and treatment cycles.

Sl. No.	Cycle No.	No. of cases followed up	0		+		++		+++	
			No.	%	No.	%	No.	%	No.	%
1.	0	15	-	-	-	-	3	20.00	12	80.0
2.	1	13	-	-	-	-	11	84.61	2	15.39
3.	2	12	-	-	1	8.33	10	83.33	1	8.33
4.	3	12	-	-	4	33.33	8	66.67	-	-
5.	4	11	-	-	3	27.27	8	72.73	-	-
6.	5	7	-	-	2	28.57	5	71.43	-	-
7.	6	6	-	-	3	50.00	3	50.00	-	-
8.	7	2	-	-	-	-	2	100.0	-	-
9.	8	2	-	-	1	50.00	1	50.00	-	-
10.	9	2	-	-	1	50.00	1	50.00	-	-
11.	10	2	-	-	1	50.00	1	50.00	-	-
12.	11	2	-	-	1	50.00	1	50.00	-	-
13.	12	1	-	-	1	100.00	-	-	-	-

In the pretreatment cycle, (80%) 12 cases showed ferning +++ and remaining (20%) 3 cases showed ferning ++.

In the 1st treatment cycle (84.61%) 11 cases had fern formarion ++ and (15.39%) 2 cases had fern formation +++.

In the 2nd treatment cycle, (83.33%) 10 cases had fern formation of ++ and (8.33%) =1 case had fern of + and +++ each.


FERN TEST ON 14/15th DAY OF CYCLE
PRE TREATMENT CYCLE
TREATMENT CYCLES
% OF CYCLES
100
90
80
70
60
50
40
30
20
10
0
0
+
++
+++
FERN FORMATION

In the 3rd treatment cycle, (66.67%) 8 cases had fern formation of ++ and (33.33%) 4 cases had ferning of +.

In the 4th treatment cycle, (72.73%) 8 cases had fern formation ++ and (27.27%) 3 cases had ferning as +.

In the 5th treatment cycle, (71.43%) 5 cases had ferning ++ and (28.57%) 2 cases had ferning +.

In the 6th treatment cycle, (50%) 3 cases showed fern formation + and another (50%) 3 cases had ferning ++.

In the 7th treatment cycle, (100%) 2 cases had ++ fern formation.

In the 8th, 9th, 10th and 11th treatment cycles, (50%) 1 case had ferning + and another (50%) 1 case had ferning ++.

In the 12th treatment cycle, 1 case (100%) had ferning +.

TABLE XXI-B : Distribution of cases according to fern test on 27th day of cycle in pretreatment and treatment cycles.

Sl. No.	Cycle No.	No. of cases followed up	0		+		++		+++	
			No.	%	No.	%	No.	%	No.	%
1.	0	15	14	93.33	1	6.67	-	-	-	-
2.	1	13	9	69.23	1	7.69	-	-	3	23.08
3.	2	11	9	81.82	1	9.09	-	-	-	-
4.	3	11	5	45.45	5	45.45	1	9.09	-	-
5.	4	10	7	70.00	-	-	1	10.00	2	20.00
6.	5	5	1	20.00	2	40.00	1	20.00	1	20.00
7.	6	5	4	80.00	-	-	-	-	1	20.00
8.	7	1	-	-	1	100.00	-	-	-	-
9.	8	2	2	100.00	-	-	-	--	-	-
10.	9	2	1	50.00	1	50.00	-	-	-	-
11.	10	2	2	100.00	-	-	-	-	-	-
12.	11	1	-	-	1	100.00	-	-	-	-
13.	12	1	1	100.00	-	-	-	-	-	-

In the pretreatment cycle, (93.33%) 14 cases showed ferning to be 0, and only (6.67%) 1 case showed ferning +.

In the 1st treatment cycle, (69.23%) 9 cases showed 0 fern formation, 23.08 (3 cases) showed +++ ferning and (7.69%) 1 case showed + ferning.

In the 2nd treatment cycle, (81.82%) 9 cases showed ferning to be 0 and (9.09%) 1 case showed ferning +.

In the 3rd treatment cycle, (45.45%) 5 cases showed ferning 0, another (45.45%) 5 cases had ferning + and (9.09%) 1 case had ferning ++.

In the 4th treatment cycle, 10 cases were followed up in which (70%) 7 cases had ferning 0, (20%) 2 cases had ferning +++ and only (10%) 1 case had ferning ++.

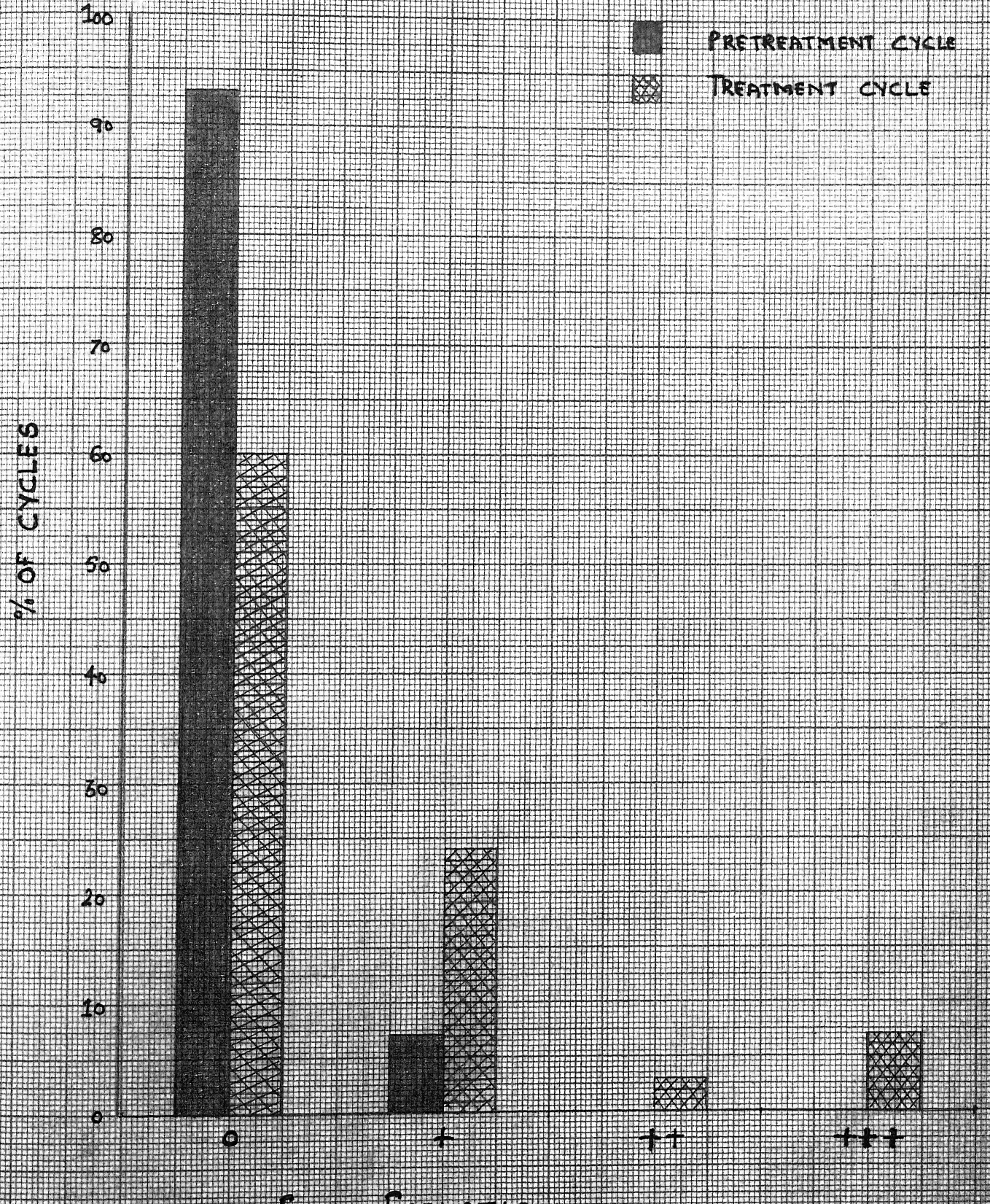
In the 5th treatment cycle, out of 5 cases, (40%) 2 cases had ferning +, (20%) 1 case each showed ferning of 0, ++ and +++.

In the 6th treatment cycle, (80%) 4 cases had 0 ferning whereas the remaining (20%) 1 case had +++ ferning.

In the 7th treatment cycle, 100% (1 case) had + ferning.

In the 8th treatment cycle also 100% cases showed 0 ferning.

FERN TEST ON 27th DAY OF CYCLE



In the 9th treatment cycle, there was no ferning in (50%) 1 case whereas (50%) 1 case had + ferning.

In the 10th treatment cycle, 100% (2 cases) had ferning nil. Similarly in the 11th treatment cycle, the only case (100%) showed the ferning +.

In the 12th treatment cycle only one case was followed and it showed no fern formation.

TABLE XXII-A : Distribution of treatment cycles according to change in KPI at 27th day as compared to KPI at 14/15 day.

Total cycle studied	Decrease in KPI		Increase in KPI	
	No.	%	No.	%
65	51	78.46	14	21.54

Out of 65 cycles, there was an increase in only 14 (21.54%) cycles.

TABLE XXII-B : Distribution of cases with an increased KPI at 27th day, in relation to cycle length.

Sl. No.	Cycle showing KPI (+) (days)	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration No.	%	Normal cycle duration or with significant change No.	%
1.	3'1'+	29	38				
2.	3'2'	29	171				
3.	4'1'	29	183				
4.	5'5'	30	37				
5.	5'6'	30	41				
6.	6'4'	31	43				
7.	7'4'	27	41	11	78.57	3	21.43
8.	7'5'	27	45				
9.	8'4'	31	33				
10.	10'1'	30	41				
11.	11'3'	30	30				
12.	12'4'	25	30				
13.	14'1'	28	29				
14.	14'3'	28	35				

+ The figures represent the patient number and the particular cycle in which the change was observed i.e. 3'1' means cycle No. 1 of case No. 3.

On analysing the data it was observed that of the 14 cycles which showed an increased KPI at 27th day, 11 cycles were prolonged when compared with the duration

of the initial cycle. Remaining 3 cycles were of a normal or near normal duration.

TABLE XXIII-A : Distribution of treatment cycles according to change in spinnbarkeit at 27th day as compared to spinnbarkeit at 14/15th day.

Total cycle studied	Decrease in spinnbarkeit test value		Increase in spinnbarkeit test value		No change	
	No.	%	No.	%	No.	%
65	50	76.92	13	20.00	2	3.08

Out of 65 cycles studied, 13(20%) cycles showed an increase in the spinnbarkeit value and there were only 3(3.08%) cases who had no change in spinnbarkeit at 27th day as compared with 14/15th day.

TABLE XXIII-B : Distribution of cases with increased value of spinnbarkeit test at 27th day in treatment cycles in relation to length of cycle.

Sl. No.	Cycle showing S.B. value	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration		Cycle duration or with insignificant change	
				No.	%	No.	%
1.	3'1'	29	38				
2.	3'2'	29	171				
3.	4'1'	29	183				
4.	5'5'	30	37				
5.	5'6'	30	41				
6.	6'4'	31	43	10	76.92	3	23.08
7.	7'4'	27	41				
8.	7'5'	27	45				
9.	8'4'	31	33				
10.	10'1'	30	41				
11.	11'3'	30	30				
12.	14'1'	28	29				
13.	14'3'	28	35				

Further analysis of the 13 cycles with increased spinnbarkeit value at 27th day showed that 10(76.92%) of these cycles were of prolonged duration when compared to initial cycle duration whereas 3(23.09) showed a normal cycle duration or an insignificant change in duration of treatment cycle.

TABLE XXIV-A : Distribution of treatment cycles according to change in ferning of 27th day as compared to 14/15th day.

Total cycle studied	Decrease in ferning		Increase in ferning		No change in ferning	
	No.	%	No.	%	No.	%
65	51	78.46	9	13.85	5	7.69

Out of 65 cycles studied, 9(13.85%) cases showed an increase in ferning.

TABLE XXIV-B : Distribution of cases with increased ferning at 27th day in relation to length of cycle.

Sl. No.	Cycle showing ferning value	Initial cycle duration (days)	Present cycle duration (days)	Prolonged cycle duration		Cycle duration with normal or insignificant change	
				No.	%	No.	%
1.	3'1'	29	38				
2.	3'2'	29	171				
3.	4'1'	29	183				
4.	5'6'	30	41				
5.	6'4'	31	43				
6.	7'4'	27	41	7	77.78	2	22.22
7.	7'5'	27	45				
8.	11'3'	30	30				
9.	14'1'	28	29				

Out of the 9 cycles showing increase in ferning at 27th day as compared to 14/15th day, 7(77.78%) cycle

had prolonged cycle duration whereas 2 cycles had a normal or near normal cycle duration.

4. ENDOMETRIAL ASPIRATION CYTOLOGY

In the pretreatment (initial cycle) 15 cases were subjected to endometrial aspiration. On cytological examination of the smears, only 3 revealed endometrial cells with a morphology suggestive of secretory phase, of the remaining smears 4 showed only endocervical cells and in the rest no cells were visualized.

Following treatment with centchroman, 31 cycles could be followed up. Here again only 10 smears revealed endometrial cells, however, the phase of the cycle could not be distinguished due to technical difficulties in smear interpretation. Of the remaining smears 5 revealed only endocervical cells and no cell was detected in the remainder.

Due to a technical drawback endometrial aspiration cytology did not provide any observations of value.

TABLE XXV : Distribution of cases according to side effects noted during treatment cycles.

Sl. No.	Side effects	No.of cases	Percentage
1.	Prolonged cycles	7	41.18
2.	Scanty menses	12	70.59
3.	Menorrhagia	1	5.88
4.	Short cycle	1	5.88

Seven (41.18%) cases showed prolonged cycles whereas 12(70.59%) cases showed scanty menses. Only one cycle (5.88%) was menorrhagic the same cycle (5.88%) was short. No other side effect was observed.

D I S C U S S I O N

D I S C U S S I O N

In the present work, the effects of centchroman a new non steroidal oral contraceptive was studied on 17 healthy female volunteers of reproductive age group of 20-35 years. 29.41% of the cases were in the age group of 20-22 years, another 29.41% were in the age group of 26-28 years and 23.53% were in the age group of 23-25 years. 52.94% of these cases were of second parity. Cycle duration in all cases prior to treatment was 25-35 days.

During treatment a total of 101 cycles were covered of which 89.11% showed a cycle length of 25-35 days. 7.92% of the cycles were of 36-45 days and 2.97% of cycles were prolonged greater than 45 days. The prolonged cycles were randomly distributed. Vaidya et al (1977) in a study of 10 cases observed an increase in cycle length in all cases, probably due to the lengthening of the collicular phase. Annual report CDRI, 1981 stated that the delay in the onset of menses was random and not confined to any one particular cycle or individual.

Duration of flow in the initial cycle was 3-4 days in 47.06% of cases and 5-6 days in 29.41% cases. Following treatment 37.62% of the cycles had a duration of flow of 3-4 days and 36.63% of the cycles had duration of 1-2 days. The duration of flow in the cycles during treatment did not present any consistant pattern.

Amount of flow remained unchanged following treatment in 61.39% of cycles. In 37.62% of the cycles

the amount decreased and in 0.99% cycles there was an increase. The changes were not restricted to any particular cycle or volunteer. Kamboj et al (1977) in human studies with centchroman found an almost equal distribution of the disturbances in the menstrual cycle amongst all age groups and therefore found it difficult to attribute this effect to centchroman.

Findings of clinical examination including the pulse and the blood pressure showed no significant changes. consistent with the findings of Dhawan et al (1977). No abnormality was found on per vaginum examination.

Changes in body weight at +3, +6 and +12 months of use were found to be statistically insignificant consistant with the results of extended phase III clinical trials conducted by C.D.R.I., Lucknow.

BIOCHEMICAL TESTS

Findings of urine analysis at +3, +6 and +12 months of use remained within normal limits.

Although most cases showed an improvement in haemoglobin levels at +3, +6 and +12 months(Prolongation of cycles and decreased flow may be a possible cause) the changes observed were statistically insignificant.

Serum bilirubin,serum GPT, blood urea, serum creatinine, all showed no statistically significant change at +3, +6 and +12 months of use.

Roy et al (1977) observed no alteration in liver and kidney function tests during centchroman administration to normospermic and oligospermic individuals.

Das et al (1977) reported that physical and laboratory tests remained unchanged following medication with centchroman.

Lipid profile which included serum cholesterol, serum triglycerides and serum high density lipoproteins - cholesterol, revealed no statistically significant difference at +3, +6 and +12 months of use. This is consistent with the findings of multicentric trials of centchroman (30 mg weekly dose) conducted by CDRI, Lucknow in which no effect could be demonstrated on serum lipid profile of human volunteers.

Ovarian size was measured ultrasonographically at 0, +3, and +6 months of drug use and the ovarian volume calculated using the ellipsoid formula :

Ovarian volume = L x AP x T x 0.5 cc where

L = Longitudinal axis, T = Transverse axis

AP = anteroposterior axis.

Volumetric determinations aid in differentiating normal from enlarged ovaries. Normal ovarian volume according to Fleischer is approximately 10 cc. According to Nicolini (1985) an ovarian enlargement above 15 cc is pathological. Mean ovarian volume as observed by Munn et al (1985) was 6.48 ± 2.90 cc (Range 2.15 to 13.84 cc). The size of the ovary may vary according to the patients'

menstrual cycle and endocrinological status.

In our study, at 0 month, smallest ovarian volume was 2.96 cc and largest was 13.76 cc with a mean of 6.05 cc which was within normal limits.

At +3 months, out of 15 patients, 11 cases showed an increase and 4 cases showed a decrease. The changes were found to be statistically insignificant.

A_t +6 months, out of 7 cases, 5 cases showed an increase in volume compared to 0 value and 2 cases showed a decrease. The changes were statistically insignificant.

On clinical evaluation of centchroman (Nityanand et al) at CDRI and K.G's. Medical College, Lucknow, revealed no significant ovarian enlargement on ultrasonography in drug users.

In our study maximum increase in ovarian volume was observed in the two cases having the two most prolonged cycles, in one case the cycle as well as ovarian size returned to normal while still on the drug use. The other case could not be followed up for ovarian volume.

Vaidya et al (1977) attributed the increase in cycle length to prolongation of the follicular phase. They concluded that the plasma total estrogens are increased due to increased steroidogenesis by ovaries which may be a stimulatory effect on the drug on the hypothalmopitutary ovarian axis or due to direct action of the drug on the ovary sensitizing it to circulating gonadotropins.

In our study too, ovarian enlargement in the two before mentioned cases could possibly be due to prolonged follicular phase, prolonged ovarian stimulation and the unruptured follicle could lead to cystic change in the ovary, being interpreted as an increase in ovarian size.

Karyopyknotic Index

During treatment with centchroman Karyopyknotic index on 14/15th day was noted to decrease as compared to pretreatment cycle. KPI on 27th day also showed a decrease in comparison to the initial (0) cycle.

This pattern of decrease reflects the anti-sterogenic property of centchroman.

Vaidya et al (1977) demonstrated a distinct antiestrogenic effect of centchroman on vaginal KPI pattern at dose levels 60 mg/week and 120 mg/week. It was observed that the antiestrogenic effect could be variable depending upon the target sites like the vagina, cervix and uterus.

Munshi et al (1977) demonstrated antiestrogenic effect in the form of a depressed vaginal KPI at 120 mg dose schedule of centchroman, inspite of markedly increased circulating total estrogens.

Changes in the maturation index were parallel to those in KPI. A decrease in KPI corresponded to fall in percentage of superficial cell and corresponding increase in intermediate cells.

A decrease in KPI on 27th day compared to 14/15th day occurred in 78.46% of treatment cycles, indicating a progestogenic effect on 27th day of cycle. In 21.54% (14 cycles) an increased KPI index on 27th day indicated lack of progestogenic effect.

Of these 14 cycles, 78.57% (11 cycles) were prlonged. In these cycles the increased KPI could be explained by the prolongation of the follicular phase with a persistance of the estrogenic effect. In the remaining 21.43% (3 cycles) which were of normal duration the raised KPI could be due to anovulation leading to persistence of estrogenic effect.

Spinnbarkeit Test

Spinnbarkeit test on 14/15th day of treatment cycles showed a decreased length of thread formation compared to corresponding values in the pretreatment cycle, reflecting the antiestrogenic effect of centchroman.

Vaidya et al (1977) observed a distinct anti-estrogenic effect on cervical mucous at 120 mg/week dose schedule of centchroman. At 60 mg/week dose cervical mucous showed improvement in some individuals.

Spinnbarkeit at 27th day when compared with that at 14/15th day treatment cycles showed a decrease in 79.92% cycles indicative of progestogenic effect.

Here again 13 cycles showed an increased spinn-ability of cervical mucous at 27th day compared to

14/15th day. Out of these 76.92% (10 cycles) were prolonged leading to persistance of estrogenic effect. 23.08% cycles were of normal duration, and anovulation could be responsible for lack of progestational effects in these cycles.

Fern Test

Ferning at 14/15th day of treatment cycles also showed a reduction compared to pretreatment cycle. No consistant pattern was found on the 27th day.

78.46% of the treatment cycles demonstrated progestogenic effect. Of the 9 cycles with increase in ferning at 27th day compared to 14/15th day, 77.78% were prolonged and 22.22% were of normal duration.

Annual report of CDRI (1981) mentioned that the cervical score in pretreatment and treatment cycles remained the same, only the day of the peak score is shifted according to the duration of the cycle.

Endometrial aspiration Cytology

Endometrial cell study did not yeild sufficient observations for any useful conclusions to be drawn.

According to Smolka findings of cytologically detectable cyclical changes in endometrial cells are rather contradictory because of technical difficulties and the findings are not uniform.

Vaidya et al (1977) observed on endometrial histology of two cases that the endometrium was thrown out of phase and was asynchronous with hormonal events.

Taking KPI, ferning and spinnbarkeit test as indirect indicators of ovulation, 77.95% of the treatment cycles provided indirect evidence of ovulation.

Vaidya et al (1977) at 120 mg/week and 60 mg/week dose schedules of centchroman deduced that ovulation did not seem to be inhibited, although it may be delayed. The contraceptive effect was mainly due to its action on cervical mucous and endometrium affecting sperm transport and implantation.

SIDE EFFECTS

In the present study the only side effects noted were prolonged cycle, scanty menses a single short cycle with menorrhogia.

Roy et al (1977) found similar symptoms in groups receiving placebo as well as the drug.

Thus centchroman was found to be well tolerated in humans.

FAILURE

Of the 17 patients studied no patient conceived during drug use. One patient conceived following discontinuation of centchroman and her pregnancy is continuing normally.

S U M M A R Y

A N D

C O N C L U S I O N

SUMMARY AND CONCLUSION

The present study was conducted in 17 healthy females of the reproductive age group to observe the effects of centchroman a new non steroidal oral contraceptive on various biochemical parameters, ovulation and ovarian size.

Centchroman was given according to 30 mg biweekly dose schedule for first 3 months and then once/week for the rest of the time. First tablet was given on the first day of menses and subsequent tablets were given every Sunday and Wednesday irrespective of the menses day or delayed menstruation. From the 4th month onwards only one tablet was given every Sunday.

Biochemical tests and ultrasonography for ovarian size was done at initial pretreatment month and +3, +6, and +12 months of treatment.

Ovulation was indirectly infered by study of the parameters of vaginal cytology, cervical mucous for ferning and spinnbarkeit and endometrial aspiration cytology. All these except the last were studied in initial pretreatment cycle during mid cycle (14/15th day) and premenstrually (27th day) and subsequently on 14/15th day and 27th day in every treatment cycle. Endometrial aspiration was done premenstrually 27th day of initial cycle and there after on 27th day of each treatment cycle.

The following conclusions were drawn :

1. Volunteers selected for the trial were 20-35 years of age with parity of 1 to fourth. Maximum patients were in the age group of 20-22 years or 26-28 years and with a parity of two.
2. Centchroman causes a delay in menstruation. Menstrual delay upto 45 days was found in 7.92% and greater than 45 days in 2.97%.

   Prolonged cycles showed no consistent pattern and were not restricted to any individual making it difficult to attribute the effect to centchroman.
3. Biochemical tests (urine analysis, haematological tests, lipid profile) all remained within normal limits on drug use.
4. No statistically significant ovarian enlargement was detected during use of centchroman.
5. KPI on 14/15th day and 27th day in various treatment cycles showed a fall as compared to corresponding values in pretreatment cycle demonstrating the anti-estrogenic effect of the drug.
6. Cervical mucous study demonstrated decreased spinnability and ferning on 14/15th day of treatment cycles compared with initial cycle, indicating antiestrogenic effect of centchroman on cervical mucous.
7. Endometrial aspiration cytology was not a sufficiently useful parameter to detect ovulation.

8. Taking KPI, spinnbarkeit and ferning as indirect indicators of ovulation 77.95% of the cycles were ovulatory.

9. The side effects observed were menstrual disturbances in the form of prolonged cycles, scanty menses, short cycle and menorrhagia.

10. There was no patient failure or method failure. One patient conceived following discontinuation of the drug and her pregnancy was continuing normally.

The antifertility action of centchroman could be due to its antiestrogenic effects on cervical mucous and vagina making it unfavourable for sperms.

A definite conclusion from this study is difficult because of limited number of patients and improper follow up.

B I B L I O G R A P H Y

B I B L I O G R A P H Y

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